VOLUME XXII MARCH, 1930 No. 2

LARVAL FLUKES ASSOCIATED WITH THE CERCARIAE OF *CLONORCHIS SINENSIS* IN BITHYNOID SNAILS IN CHINA AND ADJACENT TERRITORY[1].

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(With Plates XXIV—XXVIII.)

INTRODUCTION.

THE larval flukes described in this paper were discovered during an intensive study in China of the molluscan hosts of *Clonorchis sinensis* (Faust and Khaw, 1927). In the course of the investigation it was found that the adolescaria or encysted stage of *Clonorchis* was present in practically every species of fresh-water fish in China, Japan, Korea, Formosa and French Indo-China, and that any mammal which, either voluntarily or by accident, consumed such fish, provided the fish was not thoroughly cooked, became infected with this liver fluke. With respect to the molluscan, or first intermediate host, however, the appropriate stages of the fluke were found to be limited to a single sub-family, the *Bithyniinae* Stimpson, 1865, represented by four species in the Sino-Japanese area, viz. *Parafossarulus striatulus* (Benson, 1842), throughout China, and the variety *japonicus* Pilsbry, 1901, in Japan and Korea; *P. sinensis* (Neumayr, 1887) in the Yangtze Valley; *Bithynia fuchsiana* von Möllendorf, 1888, throughout China, but more common in the north; and *B. longicornis* Benson, 1856, distributed from Peking to Tonkin. Of these four species, *P. striatulus* has been found naturally infected in endemic foci in China and Japan, and the writer has artificially infected the two species of *Bithynia*. Although *P. sinensis* has not been actually incriminated as a host of *Clonorchis*, its presence in heavily endemic areas in the Central Yangtze Valley, where the other species are relatively uncommon, suggests that it, too, will sooner or later be found to carry this fluke.

In the course of dissection of several thousands of bithynoid snails it became clear that the cercarial stage of *Clonorchis* constituted a relatively small percentage of the total progeny of larval flukes harboured by these molluscs. It is, therefore, a matter of practical importance, as well as of theoretical interest, that these several species of larval flukes be described. Up to the present time the following six species have been studied: *Cercaria*

[1] Contribution from the Parasitology Laboratory, Department of Tropical Medicine, Tulane University of Louisiana.

bulimnorum Faust, 1924, from Shinyei, Korea; *C. benigna* Faust, 1924, from *P. striatulus*, Shinchiku, Formosa; *C. pseudoechinostoma* Faust, 1924, from *P. striatulus*, Soochow, China; *C. lactucicauda* Faust, 1924, from *P. striatulus*, Soochow, China; *C. tridonta* Faust and Nishigori, 1926, from *P. sinensis*, Anking, China, and *C. translucens* Faust and Nishigori, 1926, from *P. striatulus*, Chaochowfu, Kwangtung, China.

The present paper comprehends eleven new species, all of which were obtained in China from bithynoid snails, with the exception of one species which was dissected out of *Planorbis möllendorfi* (Peking). The general characteristics, as well as the excretory system of each species, were studied on living specimens as far as was possible, but certain structures were seen only in fixed material stained with Delafield's hematoxylin. The forms described in this paper are as follows: *Cercaria bursacetabula* n.sp., *C. lophocauda* n.sp., *C. yenchingensis*, n.sp., *C. triophthalmia* n.sp., *C. beatifica* n.sp., *C. complicata* n.sp., *C. chitinostoma* n.sp., *C. catenadena* n.sp., *C. tauiana* n.sp., *C. anhweiensis* n.sp., and *Cercariaeum sinense* n.sp.

1. **Cercaria bursacetabula** n.sp.

(Pl. XXIV, fig. 1.)

This minute xiphidio-cercaria was obtained from 32 per cent. of *Bithynia fuchsiana*, taken from the lake in Prince Tsai's Garden on the Yenching University Campus, Peking, October 15th, 1926. It is a typical virgulate larva, with a body having a broadly rounded anterior end and a truncated posterior end, and with a caudal appendage slightly shorter than the body. Both the body and tail are covered with minute integumentary spines, although only those on the anterior half of the body are long enough to be visible except with an oil immersion objective. The body measures 75μ long by 50μ in width, while the tail is 60μ long by 8μ in cross-section at its base. The oral sucker has a diameter of 20μ. On its inner dorsal wall there is a quill-shaped stylet having a length of 10μ. At the inner end of the oral cavity there is a typical *virgula*, having a cross-section measurement of 8μ; immediately behind it is a pharyngeal bulbus 6μ in diameter. Oesophagus and intestinal caeca were not developed in the cercaria.

The acetabulum, which is situated in the equatorial plane, is rounded anteriorly and truncate posteriorly. It measures 11μ in width by 12·5μ in length. Situated on its anterior aspect is a horseshoe-shaped fold, covered with coarse spines, giving a pocket-like appearance to the sucker.

The excretory system consists of a posterior bladder which is not separated from the conspicuous lateral cornua. The collecting tubules, which empty into the cornua, may be traced to the equatorial plane, where they divide each into an anterior and a posterior branch. Each of these secondary tubules, in turn, divides to form two tertiary tubules, and these last bifurcate to form the capillaries, with a single flame cell at each terminus (see Pl. XXIV, fig. 1). The flame-cell formula is, therefore, 2 [2 (1 + 1) + (1 + 1)].

There are no cystogenous glands. The histolytic glands (*e.g.* cephalic glands) consist of two groups on either side of the body, an inner cluster of about five glands reaching posteriad only to the posterior margin of the acetabulum, and an outer one of about seven glands extending to the plane of the excretory pore. These glands contain coarsely granular material which is oxyphylic in reaction. The gland ducts coalesce and open through paired pores within a bulbous terminus on either side of the stylet.

The genital primordia have not been observed.

Cercaria bursacetabula develops in an elongated oval sporocyst in the inter-hepatic lymph spaces of its hosts. Encystment has not been observed, nor is it probable in view of the absence of cystogenous glands. The further history of this fluke is unknown. It seems altogether probable, however, that it actively enters its next host.

2. **Cercaria lophocauda** n.sp.

(Pl. XXIV, figs. 2, 2 *a*.)

This extremely minute xiphidio-cercaria was obtained in a 14 per cent. infection of *Planorbis möllendorfi*, obtained in a mountain stream near Laughing Buddha Temple (Wo Fo Ssu) near Peking, September 25th, 1926. The body, which is covered with delicate spines, is broadly oval at body extremities and measures 31μ in length by 26μ in cross-diameter. The aspinose caudal appendage has a total length of 36μ and a diameter of 8μ. At its distal extremity it is provided with a fluted margin. The oral sucker measures 10·1μ in diameter and has inserted in its dorsal wall a nib-like stylet about 6μ in length. The acetabulum, which lies in the posterior third of the body and which is directed posteriad, measures 6·2μ in diameter. Within the oral cavity there is a long prepharynx, followed by a pharyngeal sphincter of 3·3μ diameter. Behind the pharynx there is a short oesophagus which leads into intestinal caeca, lined with glandular cells.

The excretory system is extremely simple, consisting of undifferentiated bladder, cornua and tubules. In the equatorial plane the primary tubules bifurcate to form the secondary tubules. These latter, in turn, bifurcate to form the capillaries, each with a single flame-cell terminal. The flame-cell formula for the species is 2 [2 (1) + 2 (1)].

There are no cystogenous glands. The histolytic glands consist of a single cell on either side of the body, with a duct opening just lateral to the stylet.

Cercaria lophocauda develops in an oval sporocyst in the inter-hepatic lymph spaces of its host. Its further history is unknown.

3. **Cercaria yenchingensis** n.sp.

(Pl. XXIV, figs. 3, 3 *a*.)

This monostomate cercaria was obtained in a 15 per cent. infection of *Bithynia fuchsiana* from Prince Tsai's Garden on the Yenching University Campus, Peking, and is named in honour of the University, which perpetuates

the ancient name of the North Capital of China. The organism is characterised by having an aspinose heart-shaped body and an extremely long caudal appendage. A pair of characteristic monostomate locomotor processes is situated on the sides of the junction of the tail with the body. The body measures 66·6μ long by 46·6μ broad, while the tail is nearly three times the body length and has a diameter of 10μ at its base. There is a pair of pigmented eye spots on the dorsal side of the body, slightly anterior to the bifurcation of the gut. Around them and between them, and to a lesser extent under the entire dorsal integument, there is a brownish pigmentation. The oral sucker measures 7·5μ in diameter. It leads into an oesophagus, which forks to form the intestinal caeca, which extend posteriad to the plane of the excretory bladder.

The excretory system consists of a bladder at the posterior end of the larva, a pair of main lateral collecting tubules, and secondary and tertiary tubules with terminal capillaries and flame cells. On account of the extreme opacity of the body, due to the cystogenous material, the more delicate portions of this system have not been definitely traced. The bladder is oval, being compressed transversely. The main tubules arise from the anterior lateral aspects of the bladder and describe a parabolic curve, just mesad to the caeca. The two tubules meet anteriad at the bifurcation of the intestine, and a short blind median pouch extends up toward the plane of the oral sphincter. The main tubules contain numerous excretory granules.

No histolytic glands have been observed, but the entire mesenchyma is packed with cystogenous cells. The cercaria develops in an elongate lenticular redia (Pl. XXIV, fig. 3 *a*). Encystment occurs soon after the cercaria escapes into the water.

4. **Cercaria triophthalmia** n.sp.

(Pl. XXV, figs. 4, 4 *a–d*.)

This cercaria was obtained from three of fifty specimens of *Bithynia fuchsiana*, taken from a brick kiln pond outside the Middle East Gate of Peking on May 9th, 1926. The body is narrowly spatulate and measures 280μ long by 140μ in greatest breadth, while the tail has a length of 300μ and a proximal diameter of 30μ. The entire body is aspinose save for four or five concentric rows around the anterior "nose" of the larva (Pl. XXV, fig. 4 *c*). There are a pair of lateral eyes and a cyclopean eye just anterior to the bifurcation of the intestine. The white centre of the latter is always exposed, while the paired eye spots are covered with pigment. Pigmentation is pronounced on the dorsum around the eye spots and delicate flecks of pigment are seen over the entire body.

The oral sucker has a transverse diameter of 30μ. It leads into a long delicate oesophagus which bifurcates at the junction of the anterior and median thirds of the body. The caeca end near the posterior extremity of the body. The excretory system as observed consists of a truncate bladder posteriorly

situated and a pair of main collecting tubules mostly in the extra-caecal fields and coalescing anteriad to form a single median blind caecum, which extends forward almost to the oral sucker. These main tubules are completely filled with minute excretory granules. The more delicate features of the system have not been seen, due to the large number of cystogenous cells which pack the mesenchyma of the larva. No histolytic glands have been seen. Upon emerging from the extremely thin-walled redia (Pl. XXV, fig. 4 *d*) the cercaria whips itself around most energetically for a while, but soon quiets down and rounds up, secreting a thick-walled cyst around itself, at the same time discarding its tail. Its further history is not known.

5. **Cercaria beatifica** n.sp.

(Pl. XXV, figs. 5, 5 *a–c*.)

This oculate distomate cercaria was obtained in a 12 per cent. infection of *Bithynia fuchsiana* outside the Chi Hua Men, Peking, May 9th, 1929. It is a large pretentious creature, cordate in shape when fully relaxed, with a caudal appendage which is provided with a broad fluted wing on either side (Pl. XXV, fig. 5 *a*). The body measures about 250μ in length by 160μ in greatest breadth, while the tail has a length of 400–450μ and a trunk diameter of 30μ. The eye spots are densely pigmented, but there is no appreciable pigmentation over the body. The entire integument is covered with thorn-like scales, while two circlets of coarser spines surround the anterior end. The oral sucker, which is directed forward, has a transverse diameter of 25μ and a length of 33μ. Internally it has a median muscular projection. The digestive system is probably not developed at this stage of the organism. The acetabulum, which has a diameter of 22μ, is situated somewhat posterior to the equatorial plane.

The excretory system consists of a posteriorly disposed bladder which has a median posterior chamber and a pair of lateral cornua, a pair of primary collecting tubules extending anteriad to the plane of the eye spots and then reflecting abruptly backward to form the secondary tubules, and the more delicate tertiary tubules and capillaries, with their flame cells. The pattern of these latter has not been worked out, due to the large amount of cystogenous material. A cluster of more than twelve acinus histolytic glands extends on either side of the median plane from the anterior aspect of the bladder anteriad past the equatorial plane. The ducts of these glands continue to the oral end where they open separately on either side of the inner dorsal wall of the oral sucker. There is a characteristic swelling of the ducts just posterior to the plane of the eye spots, and a characteristic flexure after they pierce the musculature of the oral sucker. The superficial mesenchyma of the cercaria is packed with cystogenous cells which obscure other features of the larva.

Cercaria beatifica develops in a heavily walled redia with a long rhabdocele gut. Developmental stages are shown in Pl. XXV, fig. 5 *c*. On emerging through the birth pore of the redia the cercaria sets up an active lashing movement, producing an image having an outline as indicated in Pl. XXV, fig. 5 *b*. This

activity may continue for an hour or more, but sooner or later the larva becomes quiescent, rounds up, secretes a moderately heavy thick wall around itself, discards its tail and waits for further development. In the event of a fresh-water fish being in the immediate vicinity the cercaria will attack the fish, work its way under a scale and penetrate through the skin, at times reaching the flesh. As soon as it has exhausted itself it encysts *in situ*. Without doubt this larva belongs to the family of heterophyid flukes.

6. **Cercaria complicata** n.sp.

(Pl. XXVI, figs. 6, 6 *a–c*.)

This lophocercous cercaria was found in a 14 per cent. infection of *Bithynia fuchsiana* in a brick kiln pond outside the Middle East Gate of Peking, May 9th, 1926. The body of the larva is oval in shape, measuring 375μ in length by 225μ in breadth, while the long heavy tail measures 550μ in length by 75μ in diameter at its proximal end. Both the body and the tail have a thick aspinose integument. The oral sucker, which has a transverse diameter of 72μ, opens antero-ventrad, while the acetabulum, which has approximately the same diameter, is situated just behind the equatorial plane. The posterior wall of the oral sucker is reinforced with a thickened plate of resistant material similar to the integument. A long delicate prepharyngeal tube leads inward to the pharynx, which is a small muscular organ 20μ in diameter, just in front of the middle third of the body. Oesophagus and caeca were not observed.

The excretory system consists of a small, transversely compressed bladder, situated at the posterior end of the body, a pair of main collecting tubules, arising from the anterior wall of the bladder and running outward and forward to the posterior wall of the oral sucker, and then reflected abruptly backward, and more distal delicate structures which were obscured by the cystogenous glands. The main collecting tubules are filled with excretory granules. In addition to these there is a caudal collecting tubule, having its origin on the posterior face of the bladder and extending half-way distad, where it bifurcates. The two rami open through minute pores just in front of the caudal terminus. Six pairs of histolytic glands are arranged symmetrically between the acetabulum and the pharynx. Their ducts open through the inner dorsal wall of the oral sucker. They appear at the place of emergence as minute papillae, each with a central capillary pore. The cystogenous glands consist of two types. The greater part of the mesenchyma is packed with polyhedral cells, each containing many slender rhabditiform cystogenous granules (Pl. XXVI, fig. 6*b*). In addition, there are four longitudinal columns, each consisting of twelve cystogenous cells, filled with highly refractive spherical granules.

The cercariae of this species develop in large numbers in a redia (Pl. XXVI, fig. 6 *c*) which has a well-developed pharynx and a long rhabdocele gut, with a neck thickening and locomotor processes. The redia bursts to discharge its cercarial brood, whereupon the cercariae move about sluggishly and soon

encyst. In the process of encystment the cystogenous granules "swell up" and their substance oozes out through minute pores in the integument. Since capsule formation is so rapid the tail is frequently "caught" in the secretion and gradually sloughs off rather than being cast off. The cyst (Pl. XXVI, fig. 6 *a*) when fully formed has a thick rough wall.

The combined structures of this cercaria suggest its distant relationship to echinostome flukes.

7. **Cercaria chitinostoma** n.sp.

(Pl. XXVI, figs. 7, 7 *a*.)

This cercaria was obtained in a 90 per cent. infection of *Bithynia longicornis* at Soochow on October 25th, 1924. It has an ovate body which measures 235μ in length by 120μ in breadth and is slightly constricted in the anterior pharyngeal plane. The tail is 300μ long by 40μ in transverse diameter at the proximal end. The body is covered with bristle-like spines but the tail is aspinose. The oral sucker is 40μ in trans-section and opens somewhat ventrad of anteriad. Internally it is lined with a very resistant secretion which in physical consistency resembles chitin. The oral sucker leads directly into a pharynx 27μ in diameter, behind which there is a post-pharyngeal sphincter. There follows a long oesophagus, which forks only after it reaches the posterior third of the body. The acetabulum is transversely compressed, measuring 57 by 30μ.

The excretory system of this species consists of the following parts: a transversely compressed bladder at the extreme posterior end of the body; a pair of main collecting tubules arising from the antero-lateral aspects of the bladder ascending to the pharyngeal plane, there to reflect abruptly backward as secondary tubules, which, in turn, ascend again to the pharyngeal plane; and capillaries with their terminal flame cells. Only the anteriormost pair of flame cells has been observed. Posteriorly a tubule descends from the bladder into the tail, bifurcating half-way distad and opening through short rami on each side. No histolytic glands have been observed. The cystogenous cells which pack the mesenchyma are cylindrical in shape and are arranged in longitudinal rows. Each cylinder contains a "stack" of cystogenous discs arranged in a roulette. The cercariae develop in large rediae, each having a pharynx, rhabdocele gut, a cervical annulus and a birth pore. Encystment takes place soon after the cercaria emerges from the redia.

Cercaria chitinostoma is apparently related to echinostome flukes.

8. **Cercaria catenadena** n.sp.

(Pl. XXVI, fig. 8.)

This cercaria was obtained as an 8 per cent. infection in *Parafossarulus sinensis*, obtained at Anking in October, 1924. It has an ovate body 250μ long by 150μ broad and a tail 230μ long by 45μ in diameter at its point of insertion with the body. The body is delicately spinose. The oral sucker has

a transverse diameter of 60μ and opens ventrad. It leads into a short unforked gut, which lacks a pharyngeal sphincter. The acetabulum has a diameter of 50 to 60μ.

The excretory system consists of a two-chambered bladder, main collecting tubules arising from antero-lateral aspects of the anterior bladder chamber and ascending to the posterior plane of the pharynx, where they are abruptly reflected, and secondary and tertiary tubules with capillaries and flame cells, although these latter were for the most part concealed by the cystogenous glands. The main collecting tubules are filled with coarse excretory granules. There is also a caudal tubule, with furci opening laterally near the distal end of the tail. The histolytic cells are arranged in four chained groups, one pair mesial to the main excretory tubules and anterior to the acetabulum, and one pair lateral to the main excretory tubules and extending posteriad almost to the body limit. They open through four bundles of ducts on the inner dorsal surface of the oral sucker. The cystogenous glands are delicate cells scattered throughout the sub-dermal mesenchyma. The redia is provided with a pharynx, short rhabdocele gut, cervical annulus and locomotor appendages. On erupting from the snail host the cercaria swims about for some time in the water, finally discarding its tail and encysting within a thin tough oval capsule.

This cercaria shows certain relationships to the echinostome group.

9. **Cercaria tauiana** n.sp.

(Pl. XXVII, figs. 9, 9 *a–d*.)

This fork-tailed cercaria was obtained as a 2 per cent. infection of *Bithynia fuchsiana*, taken from a brick kiln pond outside the Middle East Gate of Peking on May 9th, 1926. The body of the larva is pyriform, measuring 120μ in length by 60μ in breadth. The bifid caudal appendage has a trunk length of 150μ and a breadth of 15μ, while the furci measure 110μ in length. The body is provided with a spinose integument but the tail is aspinose. On lateral view the body (Pl. XXVII, fig. 9*a*) resembles a pear cut in half, while the tail is seen to be attached to the dorsal aspect of the rounded posterior end. At the oral end there is a powerful sucker about 90μ in transverse diameter, with its opening antero-ventrad. It leads into a short prepharyngeal tube, followed by a minute pharyngeal sphincter. This, in turn, leads into a short oesophagus, which soon forks to form the large intestinal caeca, which end blindly as swollen tubes in the plane of the excretory bladder. There is no acetabulum, but the entire venter is cupped into a suctorial organ like that of many adult holostome flukes.

The excretory system is perhaps the most interesting one of the larva. Its particular arrangement is probably of generic significance. The minute bladder is situated at the posterior end of the larva. From its anterior wall there are given off four main collecting tubules, one pair reaching laterad to the extra-caecal fields and one pair lying intra-caecally. The inner pair soon

fuse to form a single stem. Just behind the equatorial plane there is a transverse tubule which connects the outer pair with the median vessel. These main excretory trunks correspond to those of *Cercaria indica* XXXIII (Sewell, 1922, Pl. XXX, fig. 4), although the distribution of flame cells is not the same. At the junction point on each side of the lateral and transverse vessels there are given off three small secondary tubules, each of which bifurcates to form a pair of terminal capillaries with their respective flame cells. One of these pairs lies lateral to the pharynx (Pl. XXVII, fig. 9 *b*); one is situated near the lateral margin of the equatorial plane (Pl. XXVII, fig. 9 *c*), while the third lies in the plane of the excretory bladder. There are no flame cells extending into the tail, although there is a central caudal excretory vessel, connected proximally with the bladder and forking to enter each furcus and to open on the mesial margin near the furcal terminus. The flame-cell formula is 2 [2 + 2 + 2].

Cercaria tauiana develops in an elongate sporocyst with symmetrically rounded ends. No histolytic glands have been seen in this larva and cystogenous glands are not present. It seems likely, therefore, that the larva metamorphoses into the next stage within the same molluscan host.

The structure of this cercaria provides definite evidence that it is the larva of a strigeid (*i.e.* holostomate) fluke.

10. **Cercaria anhweiensis** n.sp.

(Pl. XXVII, fig. 10.)

This cercaria was obtained as a 1 per cent. infection in *Parafossarulus sinensis* at Anking, November 14th, 1924. The body is broadly oval and measures 140μ in length by 85μ in breadth. The tail trunk ranges from 200 to 250μ in length by 20 to 24μ in diameter at its proximal end, and the furci have a length of 185 to 210μ. Both body and tail are covered with spines. The oral sucker is elongated oval, with a transverse measurement of 26μ. It has an elongate outer opening on its ventral aspect. Internally it leads into a pharynx of 10μ diameter which is followed by a very short oesophagus and a small swollen undivided blind gut. A narrow elongated acetabulum is found in the mid-line in the posterior third of the body.

The excretory system has been completely traced. There is a minute posteriorly situated bladder, which gives off a pair of main lateral collecting tubules. These reach the anterior plane of the acetabulum, where each bifurcates to form a pair of secondary tubules, one tubule extending anteriad and one posteriad. Each gives rise to three capillaries with a terminal flame cell for each capillary. Of the posterior triad one element extends into the tail. The formula is, therefore, 2 [(3) + (3)]. From the bladder a main trunk vessel extends into the tail, bifurcating as the tail forks and opening at the caudal extremities through minutes pores.

This cercaria is provided with four pairs of histolytic glands situated in tandem between the acetabulum and caecal pouch and having duct openings on the inner dorsal wall of the oral sucker. In most respects this larva

resembles *Cercaria indica* I (Sewell, *l.c.* Pl. XXIX, figs. 1, 2), having an identical flame-cell pattern and histolytic glands of similar arrangement, although of different number. The digestive tract in the two species is likewise not unrelated. Both forms without doubt are strigeid larvae which utilise their histolytic glands to invade a second intermediate host where the transformation into the typical strigeid larva takes place.

11. **Cercariaeum sinense** n.sp.

(Pl. XXVIII, figs. 11, 11 *a*.)

This tail-less distomate larva was obtained as a 1·5 per cent. infection in *Bithynia fuchsiana* in Prince Tsai's Garden, Yenching University, Peking, on October 15th, 1926. The larva is broadly ovate and measures approximately 350μ in length by 240μ in breadth. The integument is completely covered with plaque-like scales in alternating rows, so that the general appearance is that of a diamond-hatch pattern. At irregular intervals there are also elongated integumentary projections. The oral sucker is oval, being transversely compressed and measuring 100 by 65μ. It leads into a short prepharynx, which is followed by a pharynx of 24μ diameter. Posterior to the pharynx is an oesophagus, which bifurcates just in front of the acetabulum to form the caeca. These latter extend around the acetabulum, where they end blindly at the sides of the bladder. The acetabulum is lenticular, having a transverse measurement of 90μ and a longitudinal one of 60μ.

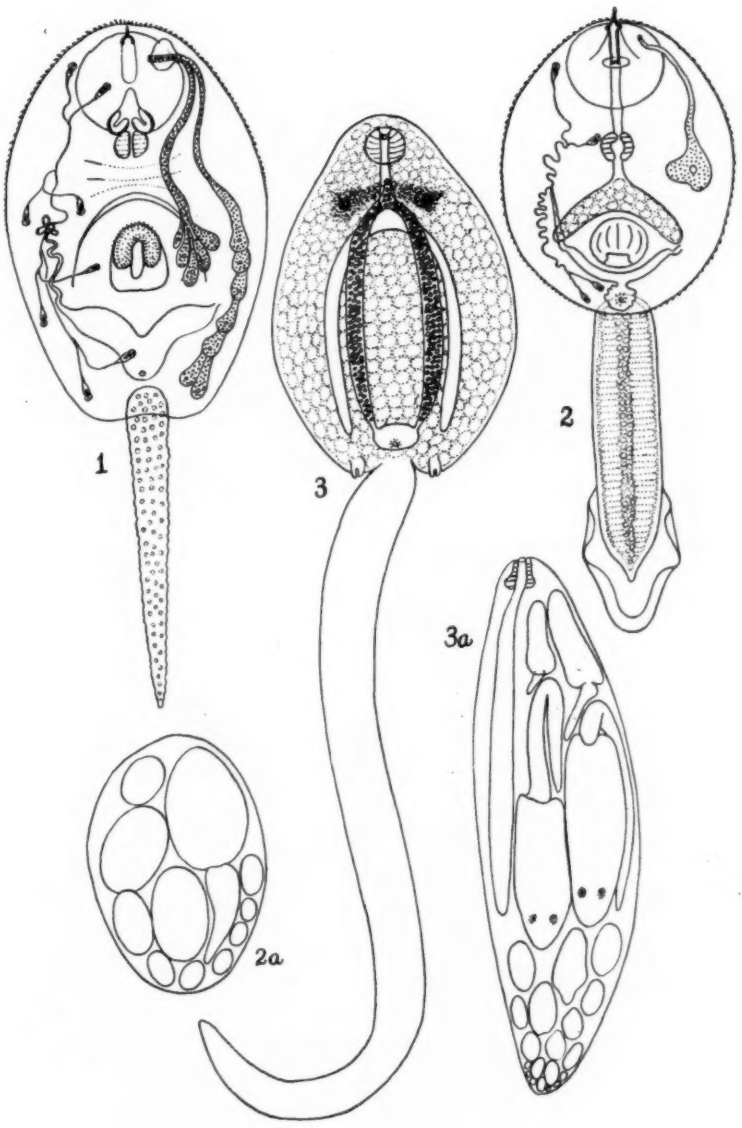
The excretory system conforms to the same general pattern as *Cercariaeum mutabile* (Cort, 1919). The posteriorly disposed bladder is longer than broad. The primary collecting tubules arise from its anterior wall, each of these tubules proceeding in a series of complicated convolutions to the plane of the pharynx, then twisting backward toward the acetabulum, where it bifurcates to form the secondary vessels. Each of these convoluted vessels then bifurcates to form the tertiary vessels. A third and yet a fourth bifurcation has been traced, so that there are eight pairs of capillaries and flame cells on either side of the body, of which four pairs are dorsal in position and four pairs are ventral. Thus the flame-cell formula is $2\,[2\,(2+2)+2\,(2+2)]$. In Cort's *C. mutabile* (*l.c.*) the formula is similar except that the capillaries and flame cells are grouped in quartets instead of in pairs. No histolytic glands have been observed.

This cercariaeum develops in a redia (Pl. XXVIII, fig. 11 *a*) having a sacculate outer structure, a pharynx and a short rhabdocele gut.

In view of its relationship to *Cercariaeum paludinae impurae* Filippi and *C. planorbis carinati* Fil. this larva may be definitely assigned to the allocreadiid flukes.

SUMMARY.

1. Eleven new species of cercariae are described from China. Of this number ten have been obtained from species of bithynoid snails and one from *Planorbis möllendorfi*.



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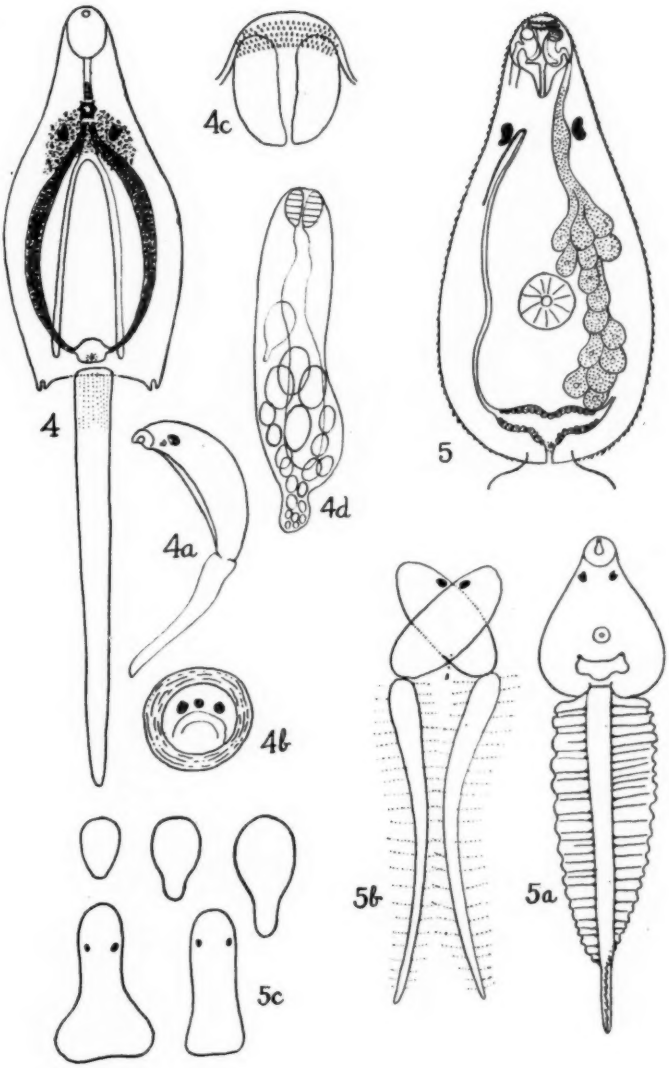
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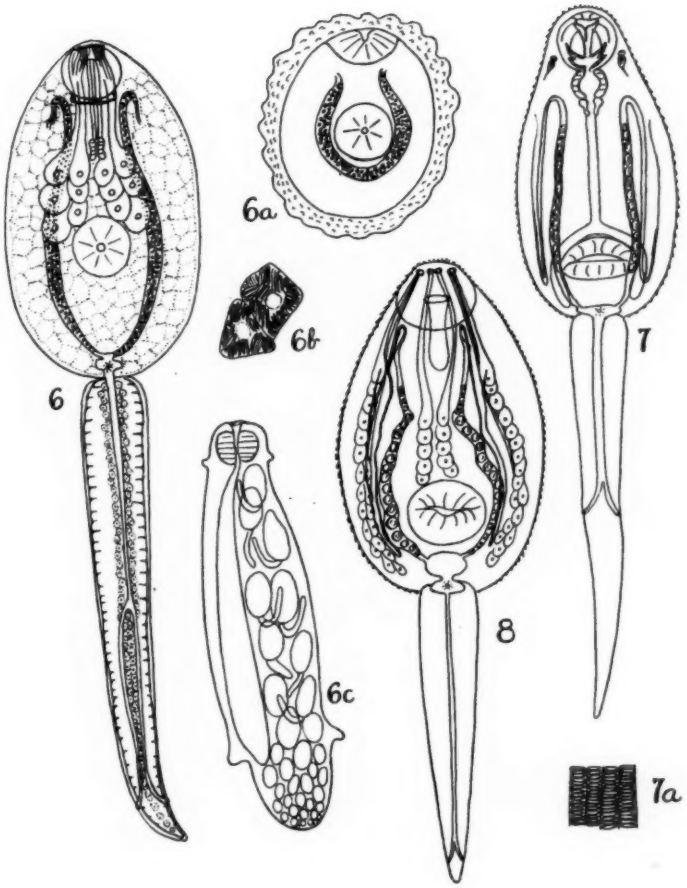

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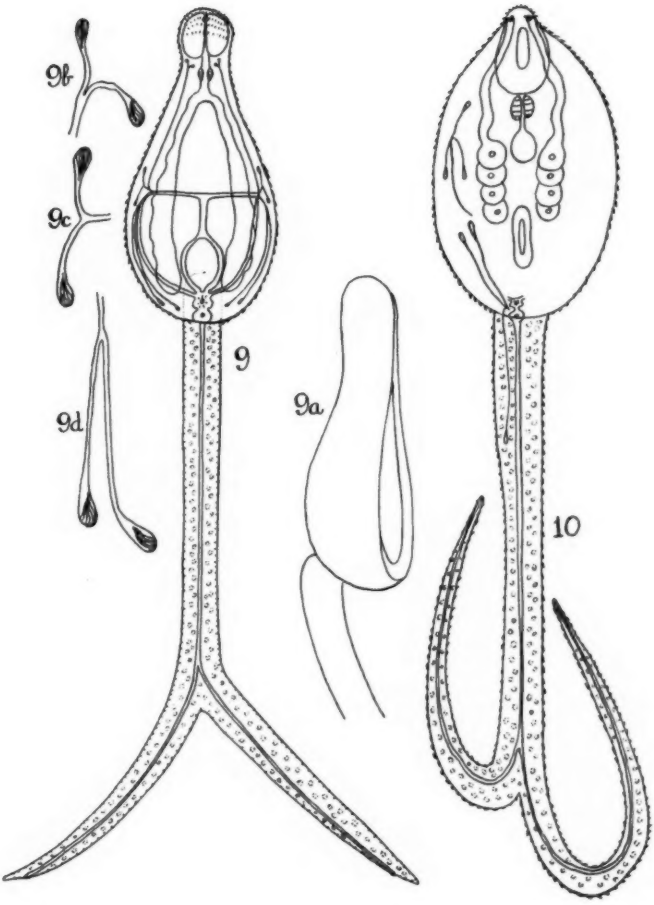

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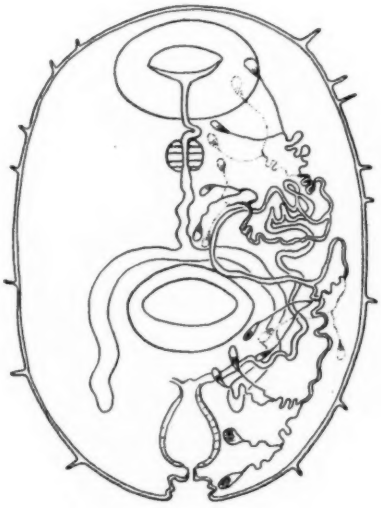

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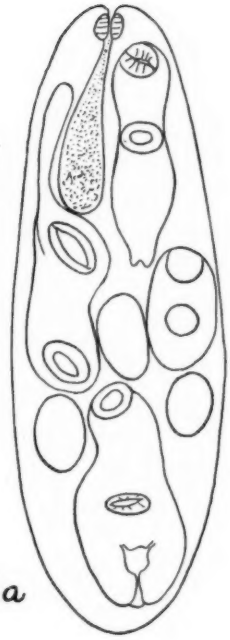

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2. One of these species, *Cercaria beatifica*, a heterophyid larva, needs to be differentiated from the cercaria of *Clonorchis sinensis*, which develops experimentally in the same mollusc.

3. The forms described are much more common parasites of bithynoid snails than is the larva of *Clonorchis*.

4. Wherever it has been possible the larval characters, particularly those of the excretory system, have been utilised to show the relationship of the cercariae to adult groups.

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DESCRIPTION OF PLATES XXIV—XXVIII.

PLATE XXIV.

Fig. 1. Ventral view of *Cercaria bursacetabula*. ×800.
Fig. 2. Ventral view of *C. lophocauda*. ×1400.
Fig. 2 *a*. Sporocyst containing immature *C. lophocauda*. ×600.
Fig. 3. Dorsal view of *C. yenchingensis*. ×800.
Fig. 3 *a*. Redia containing mature and immature *C. yenchingensis*.

PLATE XXV.

Fig. 4. Dorsal view of *C. triophthalmia*. ×200.
Figs. 4 *a–b*. Lateral view of *C. triophthalmia* and encysted larva. ×93.
Fig. 4 *c*. Enlarged anterior end of *C. triophthalmia*. ×600.
Fig. 4 *d*. Redia with immature *C. triophthalmia*. ×40.
Fig. 5. Ventral view of *C. beatifica*. ×275.
Figs. 5 *a–b*. Habit sketches of *C. beatifica*. ×100.
Fig. 5 *c*. Developmental stages of *C. beatifica*. ×100.

PLATE XXVI.

Fig. 6. Ventral view of *C. complicata*. ×133.
Fig. 6 *a*. Encysted *C. complicata*. ×133.
Fig. 6 *b*. Two cystogenous cells of *C. complicata*. Greatly enlarged.
Fig. 6 *c*. Redia with mature and maturing *C. complicata*. ×27.
Fig. 7. Ventral view of *C. chitinostoma*. ×200.
Fig. 7 *a*. Cystogenous cells of *C. chitinostoma*. Greatly enlarged.
Fig. 8. Ventral view of *C. catenadena*. ×200.

PLATE XXVII.

Fig. 9. Ventral view of *C. tauiana*. ×400.
Fig. 9 *a*. Lateral view of body and adjacent tail portion of *C. tauiana*. ×400.
Figs. 9 *b–d*. The three flame-cell pairs of the right side of *C. tauiana*. Greatly enlarged.
Fig. 10. Ventral view of *C. anhweiensis*. ×333.

PLATE XXVIII.

Fig. 11. Ventral view of *Cercariaeum sinense*. ×215.
Fig. 11 *a*. Redia with developing *C. sinense*. ×113.

(*MS. received for publication* 24. VIII. 1929.—Ed.)

SYNOPSIS OF THE GENERA AND CLASSIFICATION OF HAPLOCYTE GREGARINES.

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In a previous paper (1924) I have discussed the reasons why a division of the order Eugregarinida into Septata (Lank.) and Haplocyta (Lank.) is preferable to Cephalina and Acephalina or Polycystidea and Monocystidea. The object of the present paper is to review the genera of Haplocyte gregarines so far described, and to attempt a classification of the same into families.

Labbé (1899) in his account of the Sporozoa included in the sub-order Acephalina 10 genera and 10 "genres incertains." Minchin (1903) followed his descriptions, but considered one of the uncertain genera, viz. *Spermatophagus* Labbé as apparently a synonym of *Monocystis*. Brasil (1908) transferred the family Doliocystidae to the *Monocystids*. Hesse (1909) split up the old genus Monocystis on the basis of his work on new or already known species, and recognised two genera, viz. *Rhynchocystis* and *Stomatophora* as possessing a "true" epimerite. From the time of the publication of Hesse's work a large number of new genera have been recognised, so that the number of genera, now included in the order is 35.

As remarked by Woodcock (1926), the Aseptata have not been so completely arranged in families as the Septata. Dogiel (1909) divided the Monocystids into two families, viz. Choanosporidae and Homopolaridae, basing his classification on the form of the sporocysts alone. This classification as indicating natural affinities has certain points in its favour. As remarked by him there are two types of spores, and these are characteristic of genera, which are parasitic in marine or land forms respectively. Poche (1913) divided the tribe Monocystoidae into five families, viz. Ganymedidae J. S. Huxley, Doliocystidae Labbé, Urosporidae Woodcock, Monocystidae Bütschli, and Schaudinnellidae Poche. Neither of these classifications is entirely satisfactory, nor has it been adopted in practice. So that the writers of recent textbooks, such as Doflein (1916), Kukenthal and Krumbach (1923), Wenyon (1926) and Calkins (1926), have all overlooked or ignored the classifications of Dogiel and Poche, and contented themselves with simply mentioning and describing some of the genera of Acephalina, as if they all belonged to one and the same family. At the same time they divide the sub-order Cephalina into a number of families following Labbé's classification or some modification of the same.

As in the Septata (Cephalina), I believe the sub-division of the Haplocyta (Acephalina, Monocystidea) into families should be based on (1) the form of the gametocysts and sporocysts, (2) the shape of the trophozoite, (3) the form and character of the epimerite when present, and (4) the habitat of the parasite. I agree with Dogiel that the differences in the sporocysts are fundamental. I suggest therefore that we should divide the sub-order Haplocyta into two tribes, Heteropolaridea and Homopolaridea corresponding in the main to the Choanosporidae and Homopolaridae of Dogiel. Each of these tribes would then be divided into families, taking all the characters of the adult gregarine and the spores into consideration.

I may mention here that in 1924 I advocated that Haplocyta should be divided into two tribes, viz. Dicystidea (including forms possessing a distinct epimerite) and Monocystidea. I seemed then to attach greater importance to the presence or absence of an epimerite. But I think now that the epimerite in the Haplocyta is a variable structure, and the character of the sporocysts and the marked difference in habitat offer a more dependable basis for classification. Homopolaridea and Heteropolaridea as defined above may be regarded as two limbs of a fork, and in each there are Dicystid and Monocystid forms. Thus *Rhynchocystis* and *Stomatophora* stand in the same relation to *Monocystis* as *Lecudina* does to *Gonospora* and *Urospora*.

While the dicystid genera of Haplocyte gregarines show marked affinities with the monocystid genera, they individually show several points of contact with different families of Septate gregarines also. Thus the epimerite in the genus *Stomatophora* shows a distinct evolutionary step towards the more complex epimerite of the same type found in *Schneideria mucronata* and *Pyxinia rubecula* of the Polycystid sub-family Actinocephalinae. The form of the spores of *Stomatophora* is also intermediate between the biconical spores of *Monocystis* and the barrel-shaped ones of *Gregarina*.

Tribe I. **HOMOPOLARIDEA.**

(= Family HOMOPOLARIDAE Dogiel.)

Sporocysts with similar poles. Generally coelomic parasites of terrestrial oligochaetes.

This tribe comprises the following families:

1. Monocystidae: 4 genera.
2. Rhynchocystidae: 1 genus.
3. Stomatophoridae: 7 genera.
4. Zygocystidae: 2 genera.
5. Aikinetocystidae: 1 genus.
6. Syncystidae: 1 genus.
7. Diplocystidae: 2 genera.
8. Schaudinnellidae: 1 genus.

Family MONOCYSTIDAE s. str.

Trophozoites ovoid, spherical or elongated, without any differentiation at the anterior end, solitary. Sporocysts biconical, with similar, non-appendiculate poles, octozoic.

1. **Monocystis** Stein (1848), emended by Hesse (1909) and Cognetti (1923).

Trophozoites ovoid, without marked differentiation anteriorly, often provided at the anterior pole with a mucron, solitary. Sporocysts as defined for the family.

M. agilis F. Stein (= *M. tenax* (Duj.)) from the seminal vesicles and body-cavity of *Lumbricus agricola* Hoffm., and many other species from worms and entomostraca.

2. **Apolocystis** Cognetti, 1923.

Trophozoites spherical, without the principal axis marked by the presence of any special peripheral organ, solitary. Sporocysts as in the family.

A. lumbrici olidi (Schmidt, Hesse) from the seminal vesicles of *Dendrobaena rudiba* Sav. and *Eisenia foetida* Sav. and several other species from various earthworms.

3. **Nematocystis** Hesse, 1909.

Trophozoites elongated, cylindrical, shaped like a nematode worm, solitary. Sporocysts as in the family.

N. magna (Schmidt) from the seminal vesicles of *Lumbricus terrestris* L., and several other species from various earthworms.

4. **Rhabdocystis** Boldt, 1910.

Trophozoites elongated, gently curved, anterior end swollen in a club-shaped manner, and posterior end pointed. Sporocysts with both ends finely pointed.

Single species *R. claviformis* Boldt from the seminal vesicles of *Octolasium complanatum* Rovigno.

Family RHYNCHOCYSTIDAE fam. nov.

Trophozoites ovoid, spherical or elongated with a conical or cylindro-conical trunk at the anterior end, solitary. Sporocysts biconical, with similar, non-appendiculate poles, octozoic.

1. **Rhynchocystis** Hesse, 1909.

Trophozoites ovoid or cylindroid. Anterior pole provided with a metabolic epimerite, which is most frequently elongated into a conical or cylindro-conical trunk. Sporocysts with characters of the family.

Rh. pilosa (Cuénot) from the seminal vesicles of *Lumbricus terrestris* L., *L. rubellus* Hoffm. and *L. castaneus* Sav., and several other species from various earthworms.

Family STOMATOPHORIDAE fam. nov.

Trophozoites ovoid, spherical, cylindrical or cup-shaped, with an anterior sucker-like epimeritic organ, solitary. Sporocysts navicular, with similar, non-appendiculate but truncated poles, octozoic. All the genera and species so far described are parasitic in the species of *Pheretima* only.

1. **Stomatophora** Drzewecki, 1907, emended by Hesse (1909), Bhatia (1924).

Trophozoites ovoid or spherical. Anterior end provided with a sucker-like epimeritic organ with a central mucron. Sporocysts with characters of the family.

St. coronata (Hesse) from the seminal vesicles of *Pheretima rodericensis* (Grube), *Ph. hawayana* (Rosa), and *Ph. barbadensis* (Beddard), and several other species from various species of *Pheretima*.

2. **Astrocystella** Cognetti, 1918.

Solitary. Adult trophozoite star-shaped, on account of the presence of lobes projecting out from the central region which contains the nucleus. Anterior surface provided with a depression, myocyte peripheral.

Single species *A. lobosa* Cognetti from the seminal vesicles of *Pheretima* (*Parapheretima*) *beaufortii* Cogn.

3. **Craterocystis** Cognetti, 1918.

Solitary. Adult trophozoite sub-rotund, anteriorly provided with a sucker-like depression. Myocyte deep, well-developed, forming strong, well differentiated myonemes, directed from the concave towards the convex surface of the animal.

Single species *C. papua* Cognetti from the prostate and lymphatic glands of *Pheretima* (*Parapheretima*) *wendessiana* Cogn.

4. **Choanocystis** Cognetti, 1918.

Solitary. Adult trophozoite sub-rotund or ovoid, with an anterior organ of attachment, formed of a mobile sucker with a pseudopodial tentacle provided with cytoplasmic hairs. Myocyte deep, little differentiated in connection with the sucker.

Single species *C. tentaculata* Cognetti from the seminal vesicles of *Pheretima* (*Parapheretima*) *beaufortii* Cogn.

5. **Choanocystoides** Cognetti, 1925.

Solitary. Adult trophozoites sub-rotund or cup-shaped, with an anterior organ of attachment formed of a mobile sucker provided at the margin with cytoplasmic filaments. Myocyte deep, little differentiated in connection with the sucker.

Single species *C. costaricensis* Cognetti from the seminal vesicles of *Pheretima heterochaeta* (Mchlsn.).

6. **Beccaricystis** Cognetti, 1926.

Adult trophozoites elongated, sub-cylindrical, provided at the anterior pole with a sucker-like depression. Myocyte deep, formed of a reticular axial tube of median fibrils joined to the cuticle. Nucleus fixed in position, lying against the bottom of the sucker-like depression.

Single species *B. loriai* Cognetti from the seminal vesicles of *Pheretima* (*Parapheretima*) *sermowaiana* Cogn.

7. **Albertisella** Cognetti, 1926.

Adult trophozoite cup-shaped, provided at the anterior end with a sucker with a smooth wall. Myocyte deep, formed of fibrils directed from the concave to the convex side. Nucleus fixed in position against the bottom of the sucker.

Single species *A. crater* Cognetti from the seminal vesicles of *Pheretima* (*Parapheretima*) *sermowaiana* Cogn.

Family ZYGOCYSTIDAE fam. nov.

1. **Zygocystis** Stein, 1848.

Adult trophozoites generally pyriform, always found associated in pair or groups of three. Spores biconical with peculiar thickenings at the poles. Eight sporozoites.

Z. cometa Stein, from seminal vesicles and general body-cavity of *Lumbricus agricola* Hoffm. Several other species from Crustaceans and Oligochaetes.

2. **Pleurocystis** Hesse, 1909.

Trophozoites always associated. Association longitudinal and lateral. Spores biconical with similar poles.

P. cuenoti Hesse from *Helodrilus* (*Allolobophora*) *longus* Ude and *H.* (*A.*) *caliginosus* Savig.

Family AIKINETOCYSTIDAE fam. nov.

Trophozoites solitary or adherent, branching dichotomously, the branches being provided with sucker-like organs of attachment. Monocystid-like spores.

1. **Aikinetocystis** Gates, 1926.

Trophozoites cylindrical or columnar, with a characteristic, regular dichotomous branching at the attached end, with sucker-like bodies borne on the ultimate branches, solitary or in groups of three to eight. Spores monocystid-like. Coelomic parasites in various species of *Eutyphoeus*.

Single species *A. singularis* Gates from the coelomic cavities of *Eutyphoeus foveatus*, *E. spinulosus*, *E. rarus* and *E. peguanus*.

Family SYNCYSTIDAE fam. nov.

Spores with spines at each extremity.

1. **Syncystis** A. Schneider, 1886.

Trophozoites ovoid or pyriform. Spores boat-shaped provided with four spines at each pole.

Single species *S. mirabilis* A. Schn. from the body-cavity and fat-body of *Nepa cinerea* L.

Family DIPLOCYSTIDAE fam. nov.

1. **Diplocystis** Künstler, 1887.

Spores spherical or oval.

Trophozoites fuse precociously to form spherical masses, coelomic. Spores spherical or oblong, with eight sporozoites.

D. schneideri Künstl. from the body-cavity of *Periplaneta americana* (L.), with young stages in the intestinal epithelium. Two other species.

2. **Lankesteria** Mingazzini, 1891.

Trophozoites more or less spatulate, small size. Small epimerite described as an anterior pseudopodium-like process. Cysts spherical, produced by association of two individuals in rotation. Spores oval, with eight sporozoites. Intestinal parasites of tunicates and insects, etc.

Type species *L. ascidiae* (Lank.) from the stomach and rectum of *Ciona intestinalis*. Several other species.

Family SCHAUDINNELLIDAE Poche.

Spores spherical.

1. **Schaudinnella** Nusbaum, 1903.

Trophozoites either free in the gut or attached by an epimerite. Full-grown sporonts distinguishable as male or female. Female sporonts dividing into spherical macrogametes, and the male sporonts into a great number of minute spindle-shaped microgametes. Zygotes are passed out of the host or enter the cells of the intestinal wall and there divide into a number of sporozoites.

S. henleae Nusbaum from the alimentary canal of *Henlea leptodera* Vejd.

Tribe II. **HETEROPOLARIDEA.**

(Including Family CHOANOSPORIDAE Dogiel.)

Sporocysts with dissimilar poles. Intestinal or coelomic parasites usually of marine animals, such as polychaetes, nemertines, sipunculids, echinids and ascidians.

The tribe Heteropolaridea comprises the following families:

1. Lecudinidae Kamm: 1 genus.
2. Urosporidae Woodcock: 5 genera.
3. Ganymedidae J. S. Huxley: 1 genus.
4. Allantocystidae fam. nov.: 1 genus.

Family LECUDINIDAE Kamm.

(Nov. nom for *Doliocystidae* Labbé).

Trophozoites with an epimerite which is symmetrical, simple and deformable. Sporocysts oval, with a thickening at one pole. Habit intestinal.

1. **Lecudina** Mingazzini, 1891.

(Syn. *Doliocystis* Léger, 1893; *Ophiodina* Ming. 1893.)

Epimerite caducous or invaginable, able to take for any single species only a variety of well determined forms. Intestinal parasites of polychaetes.

Type species *L. pellucida* (Köll.) from the gut of *Nereis cultifera* Grübe and *N. beaucoudrayi* Aud. and M.E. Several other species.

As remarked by Kamm *Lecudina* was named by Mingazzini in 1891. Two years later Léger, working independently, designated a new genus *Doliocystis* for the species referred to *Lecudina* by Mingazzini. The earlier work has been overlooked by subsequent workers, and the name used by Léger has come into frequent usage.

Family UROSPORIDAE Woodcock, 1906.

(Syn. Family *Choanosporidae* Dogiel.)

Sporocysts with a typical funnel-like opening at one end.

Sub-family Urosporinae.

Epispore circular in cross-section, the funnel and its opening very distinct, and the funnel narrowed in a neck-like manner.

1. **Gonospora** Aimé Schn. 1875 emend. Pixell-Goodrich (1916 *b*). and Tregouboff (1918).

(Including *Cystobia* Ming. 1891, *Diplodina* Woodcock, 1906, *Kalpidorhynchus* Cunningham, 1907.)

Sporocysts with dissimilar poles, with a funnel at one pole and rounded at the other. Intestinal or coelomic parasites.

G. varia Léger (1892), coelomic parasite of *Audouinina* and several other species.

2. **Lithocystis** Giard, 1876 emend. Pixell-Goodrich (1916 *a*).

Elongated trophozoites, continually changing their shape and containing rapidly flowing protoplasm. Cysts spherical, gametes non-motile, slightly anisogamous. Spores ovoid, with eight sporozoites, epispore produced into a funnel at one end, and into a tubular but generally flattened tail at the other.

L. schneideri Giard and several other species from the coelome of various echinids.

3. **Urospora** Aimé Schneider, 1875 emend. Pixell-Goodrich (1916*a*).

Trophozoites elongated and coarsely granular. Somewhat sinuous movement, with slight change of outline. Anisogamy very marked, male gametes flagellated. Spores with eight sporozoites. Epispore produced at one end into a funnel, and at the other into a more or less elongated caudal filament.

U. nemertis (Köll.) from the intestine of *Nemertes delineatus* (Chiaje) and *Lineus gesserensis* (Müll.), and several other species from the body-cavities of polychaetes, nemertines, sipunculids and other marine invertebrates.

4. **Ceratospora** Léger, 1892.

Trophozoites fuse by their truncated ends and give rise to spores without encysting. Spores oval with a small hyaline collar at one extremity and the other with two elongated and rigid filaments, diverging from each other. Single species *C. mirabilis* Léger from the body-cavity of *Glycera*.

Sub-family PTEROSPORINAE.

Epispore triangular in cross-section, funnel often poorly developed, the other end of the epispore drawn out into a triangular process.

5. **Pterospora** Racov. and Labbé, 1896.

Pyriform trophozoites always associated in pairs by their broader ends, the narrow ends being provided with groups of four finger-shaped retractile prolongations. Spores with dissimilar poles, epispore with three strongly developed lateral processes.

P. maldaneorum Racov. and Labbé from the coelomic cavity of certain polychaetes.

Family GANYMEDIDAE J. S. Huxley.

1. **Ganymedes** J. S. Huxley, 1910.

The two sporonts are held together by a ball and socket-joint. Single species *Ganymedes anaspidis* in the digestive tract of *Anaspides tasmaniae*.

Family ALLANTOCYSTIDAE fam. nov.

Cysts elongated, sausage-like. Sporocysts spindle-shaped with one side slightly more prominent than the other.

1. **Allantocystis** Keilin, 1920.

Sporonts elongated, attaching to one another by their anterior extremities at the time of association, and secreting a very elongated sausage-like cyst. Sporocysts spindle-shaped, with one side slightly more prominent than the other.

Single species *A. dasyhelei* Keilin from the mid-gut of larvae of *Dasyhelea obscura* Winnertz.

Genera **Insertae sedis.**

Of the ten uncertain genera enumerated by Labbé, *Diplocystis* Künstl., *Lankesteria* Ming., *Callyntrochlamys* Frnz., *Polyrhabdina* Ming., and *Ophiodina* Ming., have since been referred to various families of Monocystids or Polycystids by various writers, and *Spermatophagus* Labbé regarded as a synonym of Monocystis. *Zygosoma* Labbé must be regarded as a genus which cannot be classified as the spores are unrecognised. *Ancora* Labbé, *Pleurozyga* Ming., *Köllikerella* Labbé, and *Lobiancella* Ming. are still in the same uncertain position. *Chlamydocystis* Dogiel, *Dirhynchocystis* Cognetti and *Grayallia* Setna must also be placed here as the spores are not definitely identified in them.

Poche has definitely referred *Diplocystis* Künstl. to Monocystidae, but I do not think it can be referred to that family, as unlike *Monocystis* and other genera included in that family, the spores are spherical or oblong and the parasites included in this genus are intestinal or coelomic parasites of insects. *Lankesteria* has been referred by Brasil and following him by Poche, to Doliocystidae, but the spores, which are oval, do not show a peculiar thickening at one pole, which is characteristic of *Doliocystis*. I think it is more closely related to *Diplocystis*, and I have consequently placed both these genera in one family under Homopolaridea. Poche has also referred *Ophiodina* to the family Doliocystidae, and Kamm has merged *Ophiodina* into *Lecudina*. Poche considers *Callyntrochlamys* as probably belonging to Gregarinoidae or Didymophidae, and I have not included it in my synopsis. Similarly Kamm has put *Polyrhabdina* in a primitive family of septate gregarines, named by her as Polyrhabidinidae.

A synopsis of the genera which cannot be classified in the present state of our knowledge.

1. **Zygosoma** Labbé, 1899.

(Syn. *Conorhynchus* Greeff, 1880 (name pre-occupied).)

Trophozoites solitary when young, always associated in the adult state, pyriform, with characteristic finger-like processes all over the body, and filled with vacuoles. Sporulation unknown.

Single species *Z. gibbosum* Greeff in the gut of *Echiurus pallassii* Guér.

2. **Pleurozyga** Ming. 1891.

Trophozoites more or less claviform, associating laterally.

P. distapliae Ming. from the intestine of *Distaplia magnilarva* Della Valle, and two other species from Ascidians.

3. **Kollikerella** Labbé, 1899.

(Syn. *Kollikeria* Ming. 1893 (name pre-occupied).)

Trophozoites of rhomboidal form, the anterior extremity rounded and forming a sort of head, separated by a constriction from the rest of the body.

Single species *K. staurocephali* (Ming.) from the intestine of *Staurocephalus rudolphi* (Chiaje).

4. **Lobiancoella** Ming. 1891.

Trophozoites of elongated form, with anterior end rounded.

Single species *L. beloneides* Ming. from the coelome of *Alciope* sp.

5. **Chlamydocystis** Dogiel, 1910.

Young trophozoites worm-like, pointed at both ends, full grown trophozoites show a rounded, stumpy anterior end, and a pointed posterior end. Trophozoites are always enclosed within the amoebocytes of the host, and never lie freely in the body-cavity of the latter. Reproductive stages and sporocysts unknown.

Single species *Ch. captiva* Dogiel from the body-cavity of *Telephus circinnatus* Fabr.

6. **Ancora** Labbé, 1899.

(Syn. *Anchorina* Ming. 1891 (name pre-occupied).)

Trophozoites with two antero-lateral prolongations, pointed or broad posteriorly, anchor-shaped, extra-cellular, fixing to the epithelial cells by a vesicle of adhesion arising from the anterior part of the body. Sexual stages and spores not recognised.

Single species *A. sagittata* (Leuck.) from the intestine of *Capitella capitata* (O. Fabr.).

7. **Dirhynchocystis** Cognetti, 1921 *a*.

(Syn. *Echinocystis* Bhatia and Chatterjee, 1925.)

Solitary. Body ovoid with two sub-cylindrical appendages, arising from opposite sides. Spores bi-conical, with similar poles.

I think Cognetti is wrong in describing the two spine-like prolongations as anterior and posterior and regarding each as comparable to the trunk-like process arising from the anterior end of *Rhynchocystis*. To me these spines suggest a strong comparison with the antero-lateral spines of *Ancora*, from which the form can be derived by shortening and rounding off of the body.

Type species *D. brasiliensis* Cogn. from the seminal vesicles of *Fimoscolex inurus* Cogn., and one other species *D. globosa* (Bhatia and Chatterjee) from the seminal vesicles of *Pheretima heterochaeta* (Mchlsn.).

8. **Grayallia** Setna, 1927.

Trophozoites Nematocystis-like, with four spine-like processes arising from the surface of the body at either end and radiating backwards, chalky-white in colour and deformable. Cysts and spores not identified.

Single species *G. quadrispina* Setna from the seminal vesicles of *Pheretima heterochaeta* (Mchlsn.).

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(*MS. received for publication* 23. VII. 1929.—Ed.)

ON CERTAIN SEMI-CARNIVOROUS ANTHOMYID LARVAE.

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(*From the Molteno Institute for Research in Parasitology, University of Cambridge.*)

(With Plate XXIX and 5 Text-figures.)

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I. Introduction.

In previous papers one of us (Keilin, 1915, 1917) has shown that among cyclorrhaphous dipterous larvae there is a remarkable correlation between the anatomical structure of the larvae and their mode of life. Although the mode of life of the larvae is in correlation with such anatomical features as thickness and hardness of the body-wall, the development of sensory organs on the head, and the structure of the alimentary canal, it is in the bucco-pharyngeal armature that the most obvious and important adaptations are to be found. The most important of these adaptations may be mentioned briefly. In certain cyclorrhaphous dipterous larvae the ventral wall of the basal sclerite of the bucco-pharyngeal armature has a number of longitudinal ridges projecting into the lumen of the pharynx. These ridges are usually Y-shaped at their free borders, and form a series of longitudinal channels in the ventral region of the pharynx. In other cyclorrhaphous dipterous larvae such ridges are absent and the ventral wall of the pharynx is smooth. This character allows the larvae to be divided into two groups—"all cyclorrhaphous dipterous larvae parasitic on the most diverse animals or on plants, as well as carnivorous larvae, and larvae which suck the blood of mammals, never have ridges in their pharynx; on the contrary, ridges are always present in saprophagous larvae" (Keilin, 1915). All the larvae which are devoid of ridges and are either parasitic, carnivorous, pass their whole life in the uterus of the female,

or are phytophagous, may be united into the group of *biontophagous*; all larvae which have ridges are saprophagous.

The structure of the bucco-pharyngeal armature permits each of these groups, biontophagous and saprophagous, to be separated into smaller groups corresponding to different modes of life. Larvae which are parasitic are characterised by the smooth ventral wall of the pharynx, and the presence of two lateral hooks without any accessory oral sclerites such as median ventral arc, oral bars and anterior and posterior ribbons. Carnivorous larvae agree with the parasitic larvae in the absence of pharyngeal ridges, but in addition to the two lateral hooks they have accessory oral sclerites in the form of median ventral arc, oral bars and anterior and posterior ribbons. Frequently carnivorous larvae have one of the lateral hooks longer than the other. Other larvae are found to have ventral pharyngeal ridges as in saprophagous forms, but the lateral hooks are often of unequal length, and there are accessory oral sclerites present such as median ventral arc, oral bars and anterior ribbon. These larvae may live either in a saprophagous manner or they may be carnivorous, and consequently they are said to be semi-carnivorous or omnivorous. Finally, there are the purely saprophagous larvae which have well-developed longitudinal ridges on the ventral wall of the pharynx, and have two lateral hooks but no accessory oral sclerites.

The object of the present work was the investigation of a number of Anthomyid larvae which were not previously described, or were not described in sufficient detail, with a view to determining whether their structure would allow them to be placed in one of the above groups, and would correspond to the known facts concerning the biology of these larvae. The larvae dealt with are: *Ophyra leucostoma* Weid. which has not hitherto been described, but which Séguy (1923) says is carnivorous. *Polietes albolineata* Fallen, and *P. lardaria* F., the biology of both of which has been well studied by Portchinsky (1910, 1911, 1913), but his anatomical descriptions, especially as regards the mouth parts, are incomplete. Portchinsky (1910) observed that *P. albolineata* is very carnivorous, while Séguy (1923) states that *P. lardaria* is also carnivorous. *Mesembrina meridiana* L. and *M. mystacea* L. are species which Portchinsky (1910, 1911) has studied fully as regards their biology, but incompletely as regards their anatomical structure. He states that *M. mystacea* is both coprophagous and carnivorous, and Séguy (1923) says that *M. meridiana* is coprophagous. The important and interesting papers of Portchinsky on these larvae are printed in Russian, and in the present paper the opportunity has been taken of giving a full résumé of his work on these larvae, concerning which there is otherwise little information in the literature.

The material on which the work is based, except in the case of *O. leucostoma*, is from the original material of Portchinsky and was obtained through the courtesy of Prof. Pavlovsky, Leningrad.

The material of *Ophyra* we owe to the kindness of Dr Graham-Smith, University of Cambridge.

II. *Ophyra leucostoma* Weid.

Séguy (1923) states that this fly is common from April to October all over Europe, the Caucasus, Syria, Tunisia and the Canary Islands. The larvae have been recorded from the carcase of a rat, a manure pit and manure in a field. The pupae were found in a swallow's nest. The larva is elongated, very much tapered at the anterior end, and truncated posteriorly. The skin is thick and hard, and the ventral surface has small spiny protuberances. It is carnivorous and devours insects which live in the same medium. The puparium is elongated, slightly tapered towards both ends; deeply segmented; and has long, thick, forwardly-curved prothoracic horns.

Graham-Smith (1916) found pupae of *O. leucostoma* about 3 inches deep beneath the soil near a manure heap on March 28 and adult flies emerged from April 4–22. This species begins to emerge when the mean temperature beneath the surface of the ground reaches 58° F., and is abundant from June to October, being most plentiful in August. It is repelled by shade and seems to be especially active in sunshine, and is seldom seen in large numbers except on sunny days. The males hover in the air like some Syrphidae. Larvae were also found in a carcase of a guinea-pig, and adults were obtained in June from human excrement and animal remains from the previous season.

1st Stage Larva. The 1st stage larva tapers towards the head and is truncated posteriorly. It is metapneustic and the post-abdominal spiracles are borne on a pair of large papillae. A small number of backwardly directed spinules are present on the ventral surface of the 3rd segment, and each of the remaining segments, 4–11, has a median ventral lozenge-shaped area bearing numerous backwardly directed spinules. The post-abdominal spiracles (Pl. XXIX, fig. 4) have long felt-chambers and open by oblique heart-shaped apertures which are guarded by inwardly directed chitinous teeth, and externally they are bordered by long, reflexed hairs. The head (Pl. XXIX, fig. 2) is provided with antennae (*an.*), maxillary palps (*m.p.*); and on each side a pair of oral grooves extend from the oral opening towards the dorsal surface of the head (*o.g.*). In front of the oral grooves there are a number of supra-buccal teeth.

The bucco-pharyngeal armature (Pl. XXIX, fig. 2) is composed of a weakly chitinised basal piece (*B.*) which is produced antero-dorsally to form the median dorsal hook (*m.d.h.*), and antero-ventrally passes into the intermediate piece (*I.*) which is then continued as the lower lip (*l.l.*). The oral opening is bordered by the 1st stage lateral hooks (*l.h.*). The anterior region of the prothoracic segment is provided with about 6 rows of backwardly directed spinules, which are mostly bifid apically.

2nd Stage Larva. The 2nd stage larva is amphipneustic and has prothoracic spiracles with 4 or 5 rays (Pl. XXIX, fig. 3). The post-abdominal spiracles have two elongated, parallel clefts, the apertures of which are guarded by long inwardly directed chitinous teeth; and there are four groups

of palmately branched hairs arising from the openings of the perispiracular glands (Pl. XXIX, fig. 5).

The bucco-pharyngeal armature (Pl. XXIX, fig. 9) is composed of a large and strongly chitinised basal piece (*B.*) the ventral surface of which has distinct longitudinal ridges (*l.r.*), while antero-dorsally the two lateral lamellae are united by a well chitinised perforated bridge-piece (*b.p.*). The intermediate piece is distinct, strongly chitinised and triangular in shape (*I.*), and articulates anteriorly with the two long slender lateral hooks (*l.h.*), which are very weak and irregular in thickness, especially near their bases. Numerous branched oral grooves radiate from the oral opening, where they unite on each side into semi-cylindrical chitinised structures (*f.*) that are approximated to form a funnel-like entrance to the oral aperture.

3rd Stage Larva. The general form of the mature 3rd stage larva is shown in Pl. XXIX, fig. 6. The anterior region of the prothoracic segment has numerous rows of backwardly directed spinules which are for the most part bifid at the apex. Each of the following three segments has a few rows of smaller, simple, spinules encircling it anteriorly. The 5th segment has a median ventral lozenge-shaped area covered with small backwardly directed spinules; and on the dorsal surface of the anterior border of this segment there are a few rows of very small spinules. Each of segments 6, 7, 8, 9, and 10 has a similar median ventral area with backwardly directed spinules; and the last, 11th, segment has two series of ventral spinules, the anterior series of which are directed forwards while the posterior series are directed backwards.

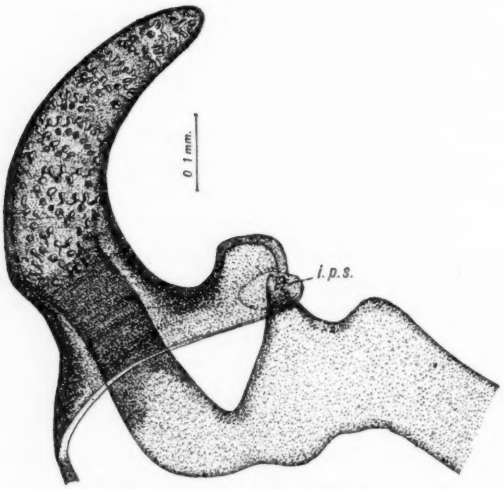
The head of the larva (Pl. XXIX, fig. 12) has comparatively large antennae (*an.*) and maxillary palps (*m.p.*); and ventral and posterior to the latter are a pair of sensory organs *C* (Keilin, 1915). Numerous branched oral grooves extend in a radiating fan-like manner from the oral aperture towards the dorsal surface of the head (*o.g.*). A pair of large labial palps (*l.p.*) with terminal sensory papillae project forwards beneath the oral opening.

The larva at this stage is amphipneustic and the prothoracic spiracles have 4 rays (Pl. XXIX, fig. 7). The post-abdominal spiracles (Pl. XXIX, fig. 8) are strongly convex and have three elongated clefts, which are arranged nearly parallel to each other, but the two outer ones converge slightly towards the oblique external scar (*e.s.*). Each cleft is curved slightly in an S-shaped manner, the curvature being most marked in the two outer ones. Four groups of palmately branched hairs radiate from the openings of the perispiracular glands.

The bucco-pharyngeal armature (Pl. XXIX, figs. 10, 11, 12) is formed of a large strongly chitinised basal piece (*B.*), the ventral surface of which bears longitudinal ridges (*l.r.*); a large and strongly chitinised intermediate piece (*I.*) into which the salivary duct (*s.d.*) opens; and two lateral hooks with accessory oral sclerites. The lateral hooks (*l.h.*) are of unequal size, one being considerably longer than the other. The relative size of the two hooks is shown in Pl. XXIX, fig. 11, where the hooks and accessory parts have been separated out. The longer

hook bears an oblique ridge (*r.*) on its internal surface with which the ventral edge of the shorter hook engages, so that both hooks work together (Pl. XXIX, figs. 11, 12). The bases of the two hooks are united ventrally by a median ventral arc (*m.v.a.*). Beneath the lateral hooks there are a pair of strongly chitinised oral bars (*o.b.*), with the antero-dorsal borders of which another pair of sclerites articulate and extend forward to unite in front of the apices of the lateral hooks and form the anterior ribbon (*a.r.*). Ventral to the oral bars on each side there is a row of cutaneous teeth (*c.t.*).

Pupa. The pupa is notable for the large size of the prothoracic horns (Text-fig. I). The horns bear numerous papillae arranged in an irregularly spiral



Text-fig. I. *Ophyra leucostoma.* Prothoracic horn of nymph. *i.p.s.* intra-pupal spiracle.

manner. Intra-pupal spiracles are present in connection with the large felt-chambers, and are formed of several groups of numerous small papillae (*i.p.s.*).

Egg. The eggs are about 1 mm. long by 0·3 mm. wide. They are slightly curved, being concave on the ventral surface, which is marked by a pair of longitudinal ridges extending from the micropyle nearly to the opposite pole. The surface is marked off into hexagonal areas (Pl. XXIX, fig. 1).

III. *Polietes albolineata* Fallen.

Portchinsky (1910) gives the following particulars about this species. During the whole summer the larvae of this fly are just as typical for the excrement of horses as the larvae of *Musca cornicina* are for the excrement of cattle. They are found isolated or in communities and very much resemble larvae of the common house fly. The body of the larva is very hard and they are very active and carnivorous. The fly is not very prolific and lays about

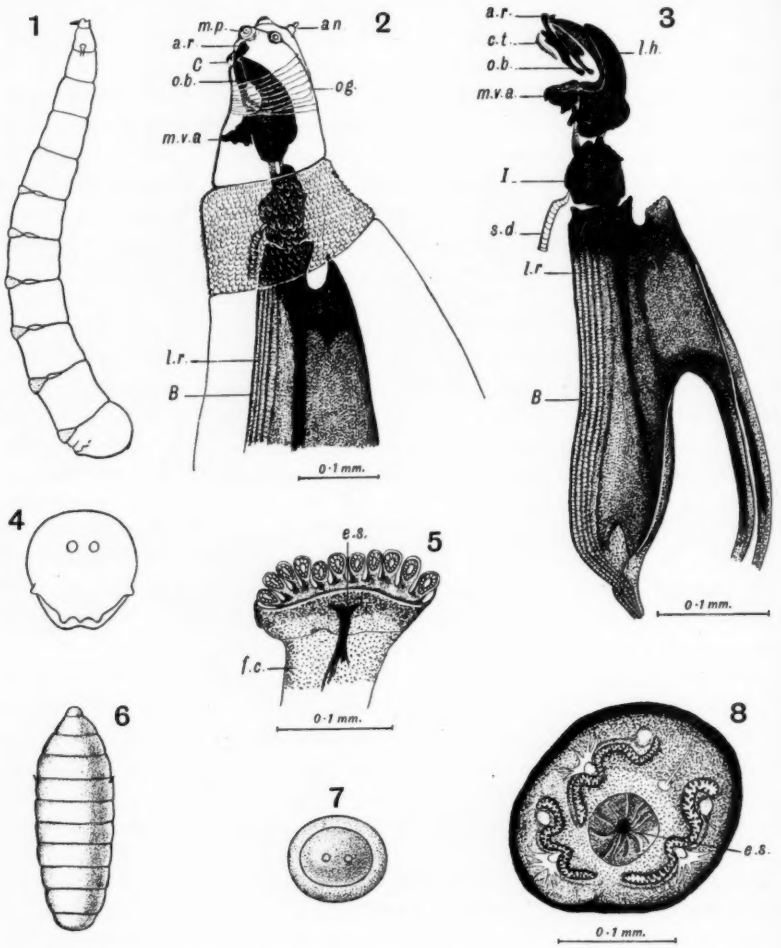
14 eggs of ordinary shape but of very large size. Larvae hatch from the eggs about one day later. From eggs collected on June 25 larvae hatched which, on June 27, were already in the 3rd stage. Puparia were formed in the middle of July and flies emerged on July 22. The body of the larva is naked. The anterior spiracles have 8–9 rays. The larva resembles that of *Mesembrina mystacea* in that the ventral protuberances begin from the anterior end of the 7th segment. This larva is the most carnivorous of all the carnivorous forms, and attacks, for instance, larvae of *Haematobia stimulans* and *Borborus*; and also attacks and destroys great numbers of larvae of the ordinary house fly, and of *Musca cornicina*, *M. ovipara*, *Hydrotaea dentipes* and *Mesembrina meridiana*.

This fly is found only in the open, and Portchinsky has never seen it or its larvae in stables. It is never found in the South of Russia. When there is not sufficient living food the larvae have to live on the fluid and semi-fluid portions of excrement, and in that case require a much longer time to reach full size. In the South the excrements are rapidly dried up, or are utilised by a large fauna which invades them, so that the larvae of *P. albolineata* would not have time to complete their life-cycle. In the North the summer is wet and there are fewer competitors among other insects for the excrements, and this is the reason larvae and flies are so very common. In the North the main enemies of this larva are crows (*Corvus cornix*) which destroy them and scatter the excrements of horses so that the larvae can no longer develop therein. It is possible that if the eggs and larvae of this fly were transported from the open into stables they would destroy on a large scale the larvae of *Stomoxys*.

Séguy (1923) states that this fly is common on flowers all over France from April to October, and is found in Vendée in August. Roubaud has seen it alight on donkeys which had just been bitten by *Stomoxys* and suck the blood running from the wounds. The larvae are very active, opaque, hard, and pale yellow in colour. They develop very rapidly in excrements of domestic animals or in decomposing material. After the 2nd stage they become carnivorous and live at the expense of larvae of *M. cornicina*, *Cryptolucilia cornicina*, *H. dentipes*, *H. stimulans*, and *Borborus equinus*. They are very carnivorous and eat each other if there is lack of food.

3rd Stage Larva. The head of the mature 3rd stage larva (Text-fig. II, 2) has prominent antennae (*an.*) and maxillary palps (*m.p.*). On the ventral surface, between the maxillary palps and the oral opening, lie a pair of sensory organs *C* (*C.*). From the oral opening a comparatively small number, about 10, oral grooves (*o.g.*) extend around the head to the dorsal surface on each side. The oral grooves diverge slightly as they extend from the oral opening and are for the most part unbranched. The anterior region of the prothoracic segment is covered with numerous rows of closely set, small spinules, which are not pointed but rounded apically.

The larva is amphipneustic, and the prothoracic spiracles have about 11 closely arranged rays and very noticeable external scars (Text-fig. II, 5). The post-abdominal spiracles are remarkable for the large external scar which



Text-fig. II. *Polietes albolineata*. (1) Mature 3rd stage larva (after Portchinsky). (2) Anterior region of 3rd stage larva. (3) Bucco-pharyngeal armature of 3rd stage larva. (4) Last segment of 3rd stage larva (after Portchinsky). (5) Prothoracic spiracle of 3rd stage larva. (6) Pupa (after Portchinsky). (7) Posterior end of body of 3rd stage larva (after Portchinsky). (8) Post-abdominal spiracle of 3rd stage larva. *an.* antenna; *a.r.* anterior ribbon; *B.* basal piece; *C.* sensory organ *C*; *c.t.* cutaneous teeth; *e.s.* external scar; *f.c.* felt-chamber, *I.* intermediate piece; *l.h.* lateral hooks; *l.r.* longitudinal ridges; *m.p.* maxillary palp; *m.v.a.* median ventral arc; *o.b.* oral bars; *o.g.* oral grooves; *s.d.* salivary duct.

has a folded appearance and is placed nearly centrally, being almost encircled by the three clefts (Text-fig. II, 8 *e.s.*). The clefts are very long, narrow and sinuous, and have their openings guarded by short, blunt, inwardly projecting

teeth. The hairs originating at the openings of the perispiracular glands are short and branched in a radiate manner.

Bucco-pharyngeal armature. The bucco-pharyngeal armature (Text-fig. II, 2, 3) is formed of a very large basal piece (*B.*) with ventral longitudinal ridges (*l.r.*). The lateral lamellae of this sclerite are deeply excavated posteriorly and are weakly chitinised except at the anterior region and for a strongly chitinised median rib on each side. The intermediate piece (*I.*) is short and very strongly chitinised. The buccal sclerites are formed of two very strongly chitinised, black, lateral hooks (*l.h.*) of which one is longer and more sharply pointed than the other. The hooks are united ventrally by a strong median ventral arc (*m.v.a.*). Beneath the hooks are a pair of strong oral bars (*o.b.*) which are toothed at their anterior ends; and, articulating with the antero-dorsal borders of the oral bars, a pair of sclerites extend forward and join in front of the lateral hooks to form the anterior ribbon (*a.r.*). Ventral to the oral bars on each side double rows of strong, sharply pointed cutaneous teeth are present (*c.t.*).

IV. *POLIETES LARDARIA* FALLEN.

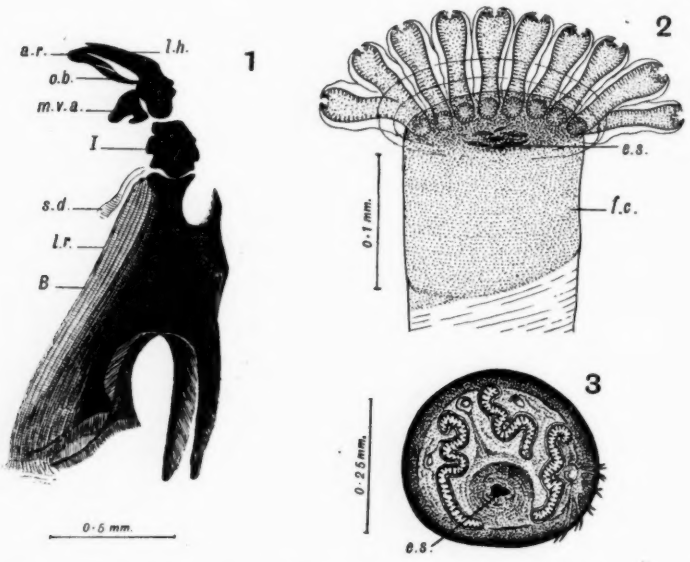
Portchinsky (1910) states that the larvae of this fly live entirely in excrement of cattle. In the North of Russia the flies usually appear in the middle of June and they are mainly found in places covered with shrubs and trees; but are also found on very fresh excrement of horse and man. In the North the females oviposit only in July or August, and only in very fresh excrement of cattle. A ripe well-developed female contains at the most 34 eggs. In August the larvae of this fly are fairly common in the excrement of cattle. The body of the larva is soft and completely naked. The lateral hooks are double. The anterior spiracles have 10–12 rays. The ventral protuberances begin on the 6th segment. The anal plate is transverse and prominent, and on it there are two papillae on each side of which there are smaller papillae; and behind the anal plate there is one more papilla which is not very noticeable.

According to Séguy (1923) the fly is common all over France from April to October, on flowers, bushes, hedges, bark of trees, along roads in woods, and on excrement. The larvae are carnivorous and develop in the same conditions as those of *P. albolineata*.

3rd Stage Larva. The mature 3rd stage larva has prothoracic spiracles with about 10 long rays (Text-fig. III, 2). The posterior spiracles resemble those of *P. albolineata* in having three long narrow sinuous clefts, but in this larva the external scar is not so large or prominent as in *P. albolineata* and is placed more towards the internal border of the peritreme (Text-fig. III, 3 *e.s.*). The apertures of the clefts have numerous short blunt inwardly projecting teeth.

Bucco-pharyngeal armature. The basal piece of the bucco-pharyngeal armature (Text-fig. III, 1 *B.*) is large, strongly chitinised, and the lateral lamellae are deeply excavated posteriorly, while the ventral surface has

numerous longitudinal ridges (*l.r.*). The intermediate piece (*I.*) is short, deep, and strongly chitinised. The lateral hooks (*l.h.*) are sharply pointed, only slightly curved, and are of unequal length. The accessory sclerites consist of a strong median ventral arc (*m.v.a.*); a pair of oral bars (*o.b.*); and, articulating with the latter, a pair of sclerites forming the anterior ribbon (*a.r.*).

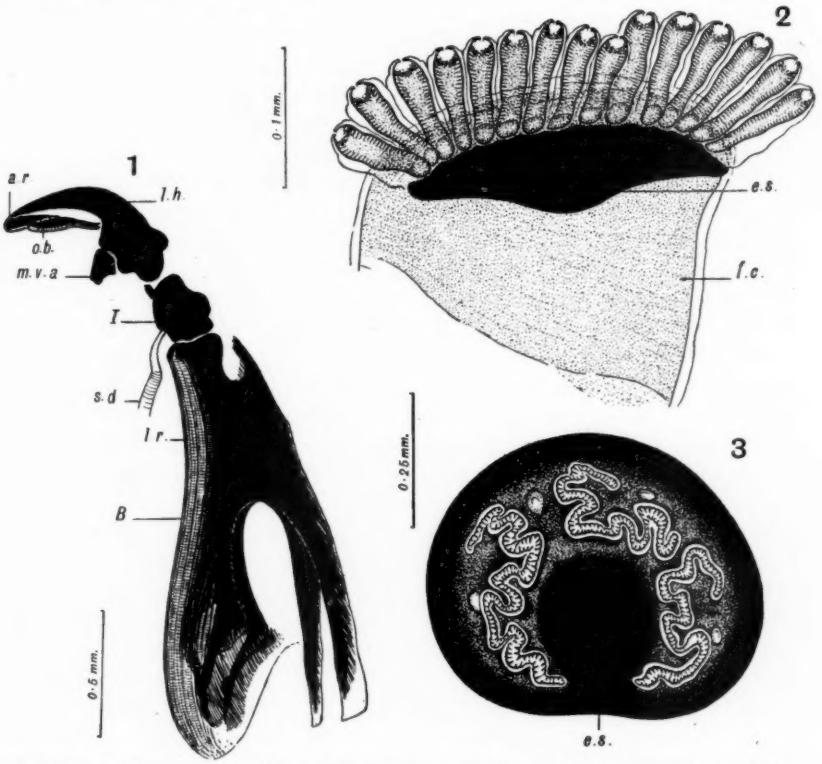


Text-fig. III. *Polietes lardaria*. 3rd stage larva. (1) Bucco-pharyngeal armature. (2) Prothoracic spiracle. (3) Post-abdominal spiracle. For lettering see explanation of Text-fig. II.

V. *Mesembrina meridiana* L.

According to Portchinsky (1910) in Russia this fly is mainly Southern; and it prefers to rest on objects exposed to the sun. In the female only three ovarian tubules are developed in each ovary, and two of these lie side by side and are the same size, while the third is very small: one of the eggs in the former two tubules already shows segments of the larva. The ripe egg passes into a specially dilated portion of the uterus where it remains for some time. When the larva is completely formed inside the egg the female deposits it in excrement of cattle, and the larva usually hatches from the egg at the moment of oviposition. Altogether this fly lays about six eggs, but deposits them separately. In captivity oviposition usually occurs about 9 a.m. The ripe egg is about 4·5 mm. long. The larvae are so adapted to excrement of cattle that they cannot live in human excrement. The first larval stage lasts only about 1–2 hours. In the 3rd stage larva the skin is almost smooth, and the anterior spiracles have 10–16 rays. The larvae are found only in very fresh excrement.

Séguy (1923) states that the adult is common everywhere from June to September, during the hottest hours of the day on flowers. Females are frequently found on excrement of ruminants. It occurs all over Europe. The female oviposits in excrement of horses and cattle a single large egg (4·5 mm.) inside which the larva is fully formed. This larva, which hatches almost immediately after it is deposited, is coprophagous and develops rapidly in the medium where it is deposited.



Text-fig. IV. *Mesembrina meridiana*. 3rd stage larva. (1) Bucco-pharyngeal armature. (2) Prothoracic spiracle. (3) Post-abdominal spiracle. For lettering see explanation of Text-fig. II.

3rd Stage Larva. The mature 3rd stage larva has anterior spiracles with about 15 long rays, closely arranged side by side, and the external scars are very large and conspicuous (Text-fig. IV, 2). The post-abdominal spiracles are very strongly chitinised and the external scar is very large and dark. The three clefts are very long, narrow and very sinuous (Text-fig. IV, 3); and there are blunt teeth projecting inwards in the apertures of the clefts.

Bucco-pharyngeal armature. The basal piece of the bucco-pharyngeal armature is strongly chitinised, deeply excavated posteriorly, and com-

paratively narrow dorso-ventrally and the ventral surface has longitudinal ridges (*l.r.*). The intermediate piece (*I.*) is short and deep and is strongly chitinised. The lateral hooks are strong and taper gradually from the base to the very sharp points (*l.h.*). The accessory sclerites are the strong median ventral arc (*m.v.a.*); a pair of oral bars (*o.b.*); and the pair of sclerites forming the anterior ribbon (*a.r.*).

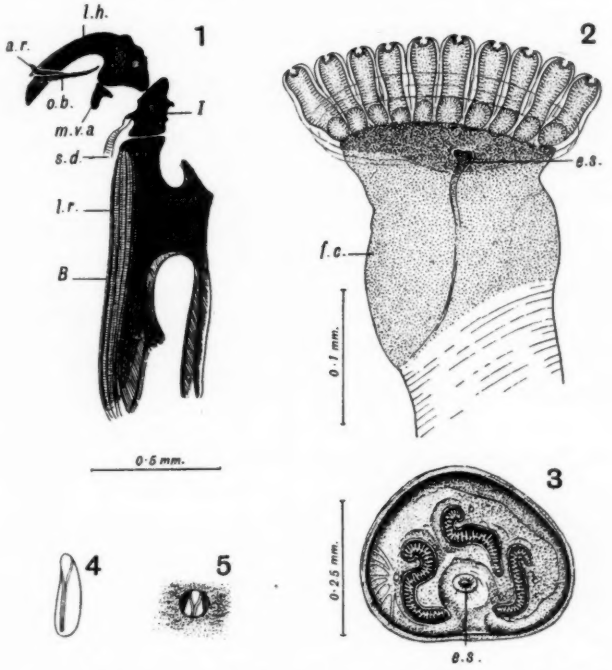
VI. *Mesembrina mystacea* L.

Portchinsky (1910) gives the following particulars about this fly. It is a representative of the fauna of the North of Russia and of high mountains. It is not very prolific, the female has not more than 18 ripe eggs at a time and usually has fewer. This means that in each ovary there are 8–9 eggs. The eggs are of gigantic size and reach 5 mm. in length (Text-fig. V, 4). The egg is introduced into excrement with its anterior end projecting (Text-fig. V, 5). Eggs deposited at 1 p.m. on August 2, for example, were found to be empty on the evening of the same day; and at 9 a.m. on August 3 the larvae were already in the 3rd stage. It is interesting to note that the larvae cannot live together as they eat each other. They also eat larvae of other species of flies such as *M. cornicina*. In other words these larvae are at the same time coprophagous and carnivorous. The 1st stage larvae resemble those of *Calliphora erythrocephala*, and are already provided with anterior spiracles, and the posterior spiracles are heart-shaped. The dorsal surface of the body is smooth and naked. The ventral surface, from the 6th to the last segment, shows bands of clear, simple, dark, spinules. The 2nd stage larva has more developed post-abdominal spiracles, each provided with two clefts. The 3rd stage larva is smooth, but the ventral projections show slight irregularities, which begin at the anterior end of the 7th segment. The second segment differs from all the other segments in having its anterior border surrounded by a wide, dark, band which is incomplete in the middle of the dorsal side. The dark colour is due to enormous numbers of small spines. The post-abdominal spiracles are of characteristic structure. The anterior spiracles have 10–13 rays. The mouth hook is well developed. Portchinsky then compares both species of *Mesembrina* in the larval stages and comes to the conclusion that they should be separated into two distinct genera.

In another paper Portchinsky (1911) gives a short description and a figure of the mouth parts of *M. mystacea*, and shows that there are two hooks of unequal size, which is a common feature of all carnivorous forms because it makes the long hook sharper than when there are two together.

3rd Stage Larva. The mature 3rd stage larva has anterior spiracles with about 10 stout rays (Text-fig. V, 2). The post-abdominal spiracles are weakly chitinised and have small eccentric external scars. The three clefts are narrow, protected by inwardly projecting teeth; and the two lower clefts are curved in an S-shaped manner, while the dorsal cleft is curved in the inverse direction (Text-fig. V, 3).

Bucco-pharyngeal armature. The basal piece of the bucco-pharyngeal armature (Text-fig. V, 1 *B.*) is comparatively small and oblong in form, and is very deeply excavated posteriorly. Longitudinal ridges are present on the ventral surface of the basal piece (*l.r.*). The intermediate piece (*I.*) is small but strongly chitinised. The lateral hooks (*l.h.*) are of unequal length and the longer one is sharply curved towards the base and is very uniform in width



Text-fig. V. *Mesembrina mystacea.* (1) Bucco-pharyngeal armature. (2) Prothoracic spiracle. (3) Post-abdominal spiracle. (4) Egg. (5) Egg as deposited in manure by female. (1), (2) and (3) of 3rd stage larva. (4) and (5) after Portchinsky. For lettering see explanation of Text-fig. II.

until it narrows abruptly to a point at the apex. There is a small median ventral arc (*m.v.a.*), and a pair of long, slender, oral bars (*o.b.*) which are dentate on the ventral borders. From the oral bars a slender anterior ribbon arises and passes in front of the lateral hooks (*a.r.*).

VII. SUMMARY AND DISCUSSION.

From the biological studies of these larvae, all except *M. meridiana* were at least suspected of being carnivorous. Séguy definitely states that *O. leucostoma* is carnivorous; while Portchinsky says *P. albolineata* is the most carnivorous of all carnivorous forms, and Séguy adds that they devour each other

if food is short. Portchinsky states that *P. lardaria* lives in excrement of cattle, but does not mention whether it is saprophagous or carnivorous, although Séguy says it is carnivorous. Both Portchinsky and Séguy regard *M. meridiana* as being purely coprophagous; while according to the former *M. mystacea* is both coprophagous and carnivorous and may destroy each other.

In all these larvae the bucco-pharyngeal armature exhibits a combination of both saprophagous and carnivorous adaptations. All of them agree in possessing the longitudinal ventral pharyngeal ridges, which are characteristic of a saprophagous mode of life; while all of them exhibit carnivorous adaptations in the presence of accessory oral sclerites consisting of a median ventral arc, oral bars and anterior ribbon; and, with the exception of *M. meridiana*, they all show an additional carnivorous character in having one of the lateral hooks longer than the other.

In the combination of saprophagous and carnivorous characters these larvae resemble certain Anthomyid larvae which have been described as being semi-carnivorous, or omnivorous, forms (Keilin, 1917). Among the latter are *H. dentipes*, which is saprophagous in the 1st and 2nd stages and then becomes carnivorous in the 3rd stage; *Muscina assimilis*, which, especially when young, may live saprophagously if living food is not available; and *M. stabulans*, which at first lives on decomposing material, but becomes carnivorous towards the end of the second instar.

The structure of the oral sclerites in *O. leucostoma*, *P. albolineata*, *P. lardaria* and *Mesembrina mystacea* is in correlation with the observed habits of these larvae, and shows that they are carnivorous. The presence of the longitudinal ventral pharyngeal ridges, however, shows that they are not purely carnivorous forms and may live also in a saprophagous manner, either in the early instars or when there is an insufficiency of living food. These larvae must therefore be considered as being semi-carnivorous or omnivorous, as are larvae of *H. dentipes*, *M. assimilis*, and *M. stabulans*. The structure of the bucco-pharyngeal armature in *M. meridiana* shows that it also is a semi-carnivorous form although it has not been observed hitherto to live otherwise than as a pure coprophage.

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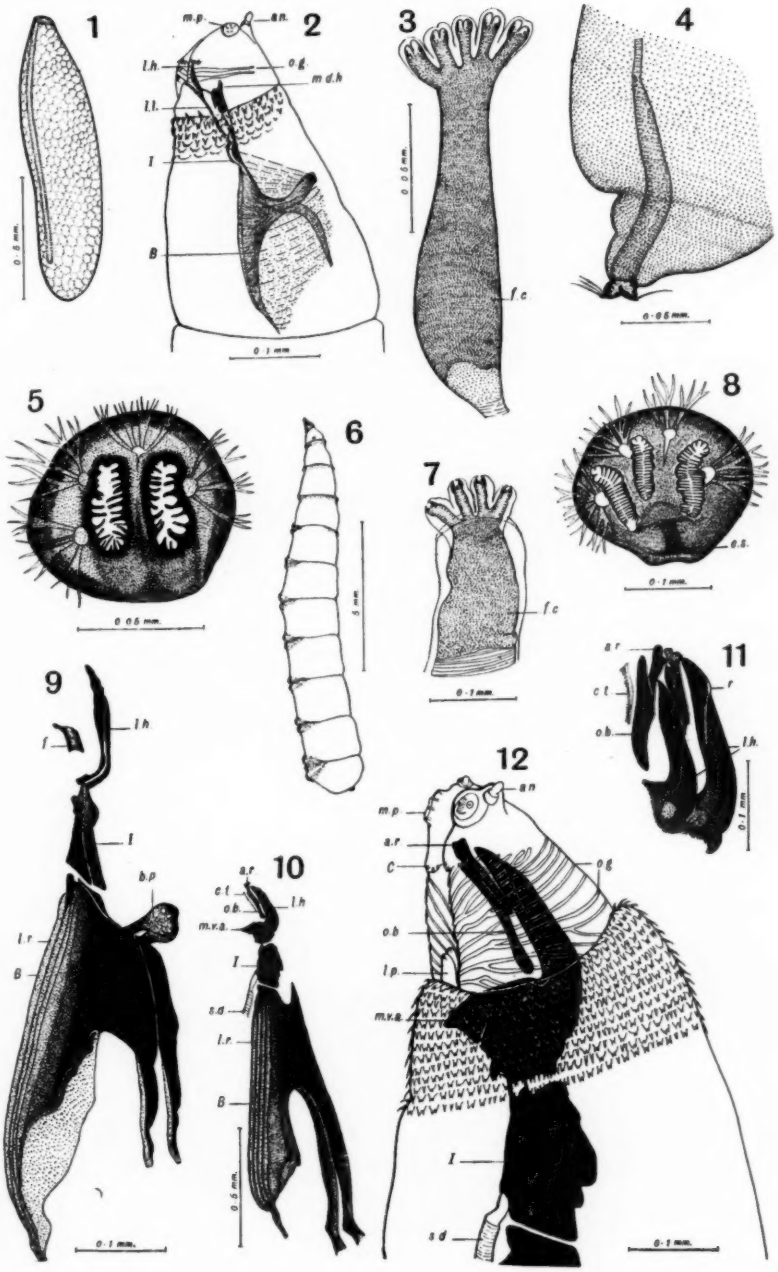
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EXPLANATION OF PLATE XXIX.

[All the figures refer to *Ophyra leucostoma*.]

Fig. 1. Egg.
Fig. 2. Anterior region of 1st stage larva.
Fig. 3. Prothoracic spiracle of 2nd stage larva.
Fig. 4. Post-abdominal spiracle of 1st stage larva.
Fig. 5. Post-abdominal spiracle of 2nd stage larva.
Fig. 6. Mature 3rd stage larva.
Fig. 7. Prothoracic spiracle of 3rd stage larva.
Fig. 8. Post-abdominal spiracle of 3rd stage larva.
Fig. 9. Bucco-pharyngeal armature of 2nd stage larva.
Fig. 10. Bucco-pharyngeal armature of 3rd stage larva.
Fig. 11. Buccal sclerites of 3rd stage larva separated out.
Fig. 12. Anterior region of 3rd stage larva.

an. antenna; *a.r.* anterior ribbon; *B.* basal piece; *b.p.* bridge sclerite; *C.* sensory organ *C*; *c.t.* cutaneous teeth; *e.s.* external scar; *f.* semi-cylindrical sclerite leading to oral opening; *f.c.* felt-chamber; *I.* intermediate sclerite; *l.h.* lateral hooks; *l.l.* lower lip; *l.p.* labial palp; *l.r.* longitudinal ridges; *m.d.h.* median dorsal hook; *m.p.* maxillary palp; *m.v.a.* median ventral arc; *o.b.* oral bars; *o.g.* oral grooves; *r.* ridge on longer lateral hook with which the short hook engages; *s.d.* salivary duct.

(*MS. received for publication* 31. VII. 1929.—Ed.)

THE CHONDRACANTHIDAE (CRUSTACEA: COPEPODA); WITH A DESCRIPTION OF FIVE NEW GENERA AND ONE NEW SPECIES.

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(With 8 Text-figures.)

Introduction.

Of all the commoner families of parasitic Copepoda, the Chondracanthidae has received perhaps the least systematic notice.

In a recent paper (1927) Leigh-Sharpe and I divided the Chondracanthidae into two sub-families, the Lernentominae and the Chondracanthinae, and gave a full description and commentary on the three genera which form the Lernentominae. The present paper is an attempt to summarise our knowledge of the remaining sub-family, and introduces five genera and one species new to science.

Historical.

The first recognisable Chondracanthid is that described and figured by Linnaeus (1747) as *Lernaea cauda duplici tereti*. A full discussion of its position and fate has appeared elsewhere (see Leigh-Sharpe and Oakley, 1927).

Muller (1777) figured six animals said to belong to the genus *Lernaea*. His figures are all small, but three of them are easily recognisable as Chondracanthids. They are *L. cornuta*, *L. gobina*, and *L. radiata*. The first belongs to the genus *Acanthochondria*, the second is the solitary species of the genus *Diocus*, and the third is a *Chondracanthus*.

Holten (1802) described *C. merluccii* as *L. merluccii*.

Delaroche (1811) described and gave an excellent figure of *Chondracanthus zei*, which thus becomes the type of the genus.

Oken (1815) erected the genus *Anops*, with type *A. radiata*. As this is a *Chondracanthus*, the name *Anops* becomes a synonym.

Krøyer (1863) added the genera *Blias* and *Diocus*, both of which are valid. All species described since that date have been thrown pell-mell into the genus *Chondracanthus*. An attempt has been made here to separate the genus *Chondracanthus* into its component genera, and for that purpose five new genera have been erected: *Acanthochondria*, *Acanthochondrites*, *Chondracanthodes*, *Chondracanthopsis* and *Disphaerocephalus*. The descriptions of *Chondracanthodes* and *Chondracanthopsis* were communicated to me in manuscript by Wilson.

OECOLOGY.

Both sexes in the Chondracanthidae are permanently fixed parasites; the female is attached to her host, usually to the gill arches or gill filaments, by her uncinate second antennae; the male is attached to the female in a like manner. As a consequence both sexes show marked degeneration, both in general shape and in loss of appendages.

Prehension. Attachment is in all cases effected by means of the second antennae. The thoracic legs of *Blias* are stated to be prehensile. In one subfamily, the Lernentominae, there is a secondary attachment mechanism, that of burying the head in the tissues of the host.

Hosts. This family is confined to salt-water fish, and above all to fish which frequent the bottom. They commonly occur on the gills and gill arches, but *C. lophii*, often in enormous numbers, almost always occurs in the opercular angle; *C. zei* in similar positions. The genus *Acanthochondria* displays a special preference for flat-fish; *Chondracanthus* is more cosmopolitan, but the species are almost confined to single hosts. The other genera have each but a few species, and are fairly local.

Food. From their presence on or near the fishes' gills, and the structure of the mouth-parts, which are adapted rather for biting than sucking, it is probable that they live on a mixed diet of flesh and blood.

MORPHOLOGY: FEMALE (Fig. 6 *A*).

General body form. Variable, sometimes the same width for the whole length, sometimes wider posteriorly. Usually divisible into four portions: a cephalothorax, composed of cephalon and one thoracic segment, sometimes provided with cephalic barbs; a free thorax, usually the second thoracic segment, often provided with dorsal processes; a genital segment, formed of the remaining thorax segments fused, with, almost constantly, posterior processes, sometimes ventral and lateral processes. Abdomen unsegmented.

Appendages. There are seven pairs (Fig. 2 *A*).

First antennae: blunt, one- to three-jointed, armed with a few setae.

Second antennae: two-jointed strongly uncinate, except in *Triphyllacanthus*, where they are blunt and tripartite.

Mandibles: two-jointed, falciform, toothed along both margins, with large palp. Both upper and lower lips are free, the upper the larger, and the mouth is not suctorial.

First maxillae: absent.

Second maxillae: two articled, provided with teeth on the inner margin, or on both margins.

Maxillipedes: three-jointed, claw-shaped, toothless.

Swimming legs: two pairs, one pair or absent (*Diocus*, *Medesicaste*). Usually clumsy, unjointed, bipartite structures, obviously vestigial and useless. They are covered with small papillae, and may less commonly be tripartite or undivided. There is usually a pair of small unarmed *abdominal appendages*.

MORPHOLOGY: MALE (Fig. 1 *D*).

Male. Relatively very small. ♀ : ♂ = 15 (average). Head fused with first one or two thoracic segments, remaining four or five free; abdomen small, unsegmented. Appendages like those of female, except that the first antennae are cylindrical, one to three articled; second maxillae are toothed in *Acanthochondrites* and *Chondracanthopsis* only; thoracic legs lamelliform, absent in *Pseudochondracanthus*. Permanently attached to female by uncinate second antennae.

The males are much more like one another than are the females, so that one male can usually be distinguished from another only by a subtle difference of body form. The characteristic form is however so constant that figures of all available males are given.

SYSTEMATIC POSITION.

Sars (1904) in his great work on the Crustacea of Norway, first offered a fairly satisfactory working classification of the Copepoda, basing it on a mixture of morphology and habits. By this means he was led into the error of grouping together under the name Lernaeoida the families Lernaeidae, Lernaeopodidae and Chondracanthidae. The only reason for this grouping, as far as one can judge, is that both sexes in the Chondracanthidae and Lernaeopodidae, and the female in the Lernaeidae, are fixed and degenerate parasites. Morphological considerations, necessarily of much greater importance in classification than habits, immediately dissolve this assumed relationship. Wilson, by his study of the development of the Lernaeidae, was forced to the conclusion that they belonged to the Caligoida, and accordingly transferred them to that group, calling the remaining forms the Chondracanthidae and Lernaeopodidae the Lernaeopodoida.

Vogt (1877) in a study of the mouth-parts of the Chondracanthidae, Ergasilidae, and Corycaeidae, came to the conclusion that the Ergasilidae were the parasitic forms corresponding to the free-swimming Corycaeidae, and that the metamorphosis had reached its lowest level in the Chondracanthidae. Wilson (1911), considering the Ergasilidae, confirmed this view, and suggested that the Ergasilidae and Chondracanthidae should be placed close together.

I suggest that the family Chondracanthidae be transferred from the Lernaeopodoida to the Cyclopoida, the change being wholly justified by comparison of the mouth-parts, and the condition of the male. With the Chondracanthidae should be transferred Wilson's as yet unlocated family Juanettiidae. Further the Lernaeopodidae are by their development, the presence of a frontal filament, their styliform mandibles and mouth-parts generally, closely related to the Caligoida, and thither, together with the Choniostomatidae, and probably the Herpyllobiidae, they must be transferred. This leaves two groups only, the Lernaeoida of Sars having completely vanished. As these two groups contain families very different from those included by Sars, I suggest the following new names:

Cyclopiformes. One or both sexes cyclopoid, that is, inflated, with non-suctorial mouth, simple or falciform mandibles, relatively small genital segment, no frontal filament. Free-swimming, commensal or parasitic.

To include: all free-swimming forms (probably), Ergasilidae, Juanettiidae, Chondracanthidae and Monstrillidae.

Caligiformes. One or both sexes or larva caligoid, that is dorsi-ventrally flattened, with suctorial mouth tube, styliform mandibles, large genital segment, frontal filament present except in the Euryphorinae. All parasitic.

To include: Caligidae, Dichelesthiidae, Lernaeidae, Lernaeopodidae, Choniostomatidae, and Herpyllobiidae. This matter will be considerably amplified in a future paper.

Family **CHONDRACANTHIDAE** Milne Edwards.

Rejected genera.

Splanchnotrophus. S. gracilis Hancock and Norman, 1900, differs from the typical Chondracanthidae in that it is parasitic on a mollusc; the genital segment is extremely small compared with the cephalothorax, which shows no signs of sub-division; the appendages are extremely small, and are by no means as degenerate as is usual in the Chondracanthidae. However, the arrangement of the eggs in the reproductive apparatus is exactly similar to that in the Chondracanthidae, so that the systematic position remains a puzzle. If a member of the present family, it must certainly be placed in a separate sub-family, the Splanchnotrophinae; one would scarcely like to make it the type of a new family without more evidence than is available at present.

Strabax. Wilson (1917) evidently intended to transfer this genus to his new family Sphyriidae, but was doubtless deterred by the typical Chondracanthoid facies of the male. I feel that in the absence of definite information, for so far as I am aware, only one undamaged female specimen has ever been reported (Nordmann, *S. monstrosus*, 1864), it is better to leave the genus where it is, while recognising the extreme doubtfulness of its position.

Tanypleurus. T. alcicornis Steenstrup and Lütken. The main feature of interest is the attachment organ, which in the smaller forms consists of two arms attached to a cirrhose bulla. In larger and therefore presumably older specimens, the arms are fused. Wilson has suggested that *Tanypleurus* probably belongs to the Lernaeopodidae, comparing the attachment organ to the second maxillae and bulla of that family; and while there is no embryology to make the matter certain, it seems better to displace the genus from the Chondracanthidae, where it certainly does not belong, to a family where its inclusion is probably justified.

Trichthacerus. T. peristedii Krøyer, 1863, obviously does not belong to the Chondracanthidae; its appendages, particularly those of the thorax, are wholly aberrant. On the other hand *T. molestus* Heller, 1865 (which does not belong to the genus *Trichthacerus*, as is evident on comparison of the authors'

figures), is certainly a member of the Chondracanthidae, and is here included as the new genus *Triphyllacanthus*.

Sub-family **Lernentominae.**

Diagnosis. Head relatively small, composed of first two cephalic segments, provided with lateral expansions, separated from remainder by a cephalic neck; antennae separated from mouth-parts, mouth at base of neck.

Parasitic on *Trigla* spp.

(For full account of this sub-family see Leigh-Sharpe and Oakley, 1927.)

Sub-family **Chondracanthinae.**

Diagnosis. Head large, composed of one thoracic segment fused with cephalon, without lateral expansions (except in *Disphaerocephalus*), no cephalic neck; antennae not markedly separated from mouth-parts; mouth on ventral surface of the head.

Not parasitic on *Trigla* spp.

Genus **Chondracanthus** Delaroche, 1811.

Female. Chondracanthinae with cephalic barbs, dorsal processes, and ventral processes all present. Thoracic legs bipartite or tripartite.

Male. Very small. ♀ : ♂ = 20 (average).

Type species. *C. zei* Delaroche, 1811.

The general features of the members of this genus have been well described from time to time, for the parasites are large and common; so that only appendages and males, which have hitherto received little or no attention, need be considered.

Cephalic barbs are postero-lateral projections of the head; *dorsal processes* are always present on the second thoracic segment, and are homologous with the dorsal processes of the Lernentominae; *ventral processes* are ventral projections from the genital segment, pointing posteriorly.

Rejected species.

The following species have already been transferred by me (1927) to the genus *Acanthochondria*: *clavata, cornuta* (type), *depressa, flurae, limandae.* To these must now be added *chilensis* Wilson, *crassicornis* Steenstrup and Lütken, *chilomycteri* Thomson, *cynoglottidis* A Scott, *epachthes* Wilson, *galeritus* Rathbun, *phycidis* Rathbun, *palpifer* Wilson, *psetti* Krøyer, *sicyasis* Krøyer, *sixteni* Wilson.

C. annulatus. Both sexes differ so greatly from the type that they are here made the type of the new genus *Acanthochondrites.*

C. nodosus. Wilson, in a manuscript note addressed to me, has adduced reasons for making this the type of a new genus *Chondracanthopsis.* Here probably belongs *C. cottunculi* Rathbun.

C. ornatus. Differs from the rest of the Chondracanthinae in possessing lateral expansions; its second thoracic legs point anteriorly. For these reasons it is made the type of the new genus *Disphaerocephalus*.

C. triglae Cuvier (published as *La Condracanthe du grondin*) is *Oralien triglae*. *C. gurnardi* ascribed by Steenstrup and Lütken (inaccurately) to Krøyer, is a synonym of *Lernentoma asellina*.

There are therefore 12 species of *Chondracanthus*: *alatus* Heller, 1865; *angustatus* Heller, 1865; *distortus* Wilson, 1902; *horridus* Heller, 1865; *genypteri* Thomson, 1889; *lophii* Johnston, 1836; *lotellae* Thomson, 1889; *merluccii* (Holten, 1802); *pinguis* Wilson, 1912; *radiatus* (Muller, 1777); *stramineus* Wilson, 1923; *zei* Delaroche, 1811.

Chondracanthus zei Delaroche, 1811.

Female. Head about as long as wide, cephalic barbs distinct; dorsal processes one pair; ventral processes two pairs tridigitate; egg-strings flattened laterally, expanded dorsi-ventrally.

Appendages (Fig. 1 *A*). First antennae large, indistinctly jointed; mandibles two articled, with terminal article a broad falciform lamina, with long base of attachment; second maxillae with slender uninflated basal article, terminal article same length as basal, provided with one terminal and thirteen lateral teeth; maxillipedes small, basal article inflated in centre, second article same length as basal, much more slender, capped with spines, terminal article very small claw. Thoracic legs two pairs, tripartite, small.

Upper lip an unindented flap. Lower lip very small.

No **males** available for description. Parasitic on gills and in opercular angle of *Zeus faber*. Almost always present.

Chondracanthus lophii Johnston, 1836.

Female. Head small, cephalic barbs distinct; dorsal processes two pairs, on first and second thoracic segments; on second thoracic segment only in young forms. Ventral processes large, undivided.

Appendages (Fig. 1 *B*). First antennae indistinctly two articled, basal inflated, terminal narrowed; mandibles long and slender, with small teeth; second maxillae with very stout basal article, lateral teeth on terminal article numerous; maxillipedes strong, basal and second article equal in length, terminal half that length, strongly curved posteriorly. Thoracic legs large, bipartite. Upper lip a notched crescent.

Male (Fig. 1 *D*). Very slender, not much curved on itself; cephalon not much inflated, second thoracic segment much the largest.

Appendages (Fig. 1 *C*). First antennae small indistinctly three articled; mandibular palp elongated with two terminal setae; second maxillae with enormously inflated basal article, terminal bottle-shaped shorter than usual, basal spine equal to it in length. Maxillipedes very long and slender. Thoracic legs laminate, first pair simple, second pair with lateral protuberance and two setae.

Parasitic on the gills and in the opercular angle of *Lophius piscatorius*; nearly always present, in large numbers.

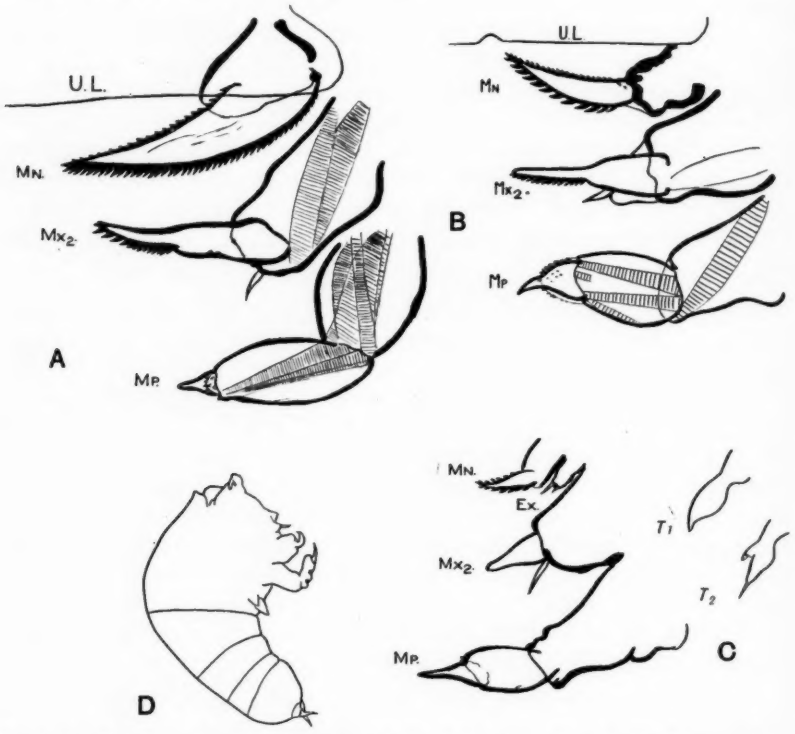

Fig. 1. A = *Chondracanthus zei*, mouth-parts of ♀; B = *C. lophii*, mouth-parts of ♀; C = appendages of ♂; D = lateral view of ♂. *Mn.* = mandible; *Mx*$_2$ = second maxillae; *Mp.* = maxillipede; *T*$_1$ = first thoracic leg; *T*$_2$ = second thoracic leg; *Ex.* = Exopodite; *U.L.* = Upper lip; throughout figures.

Chondracanthus merluccii (Holten, 1802).

Female. Head much elongated, so that antennae are somewhat separated from mouth-parts; cephalic barbs small, dorsal processes one pair small, ventral processes large.

Appendages (Fig. 2 *A*). Mandibles elongated, teeth relatively large and few; mandibular palp small, bilobed, with medial and posterior spines; second maxillae uninflated, terminal article provided with seven widely spaced large teeth; basal spine very small.

Maxillipedes large, with very long broad basal article.

Upper lip an indented flap. Thoracic legs large bipartite.

Male (Fig. 2 *C*). Head much inflated and pointed anteriorly, remaining segments tapering somewhat.

Appendages (Fig. 2 *B*). First antennae small, unjointed; mandibular palp large with two terminal spines; second maxillae with long inflated basal article, short basal spine. Maxillipedes with two proximal articles of equal length and width. Thoracic legs laminate, second pair with terminal spine.

Parasitic on the gills of *Merluccius vulgaris*; fairly common.

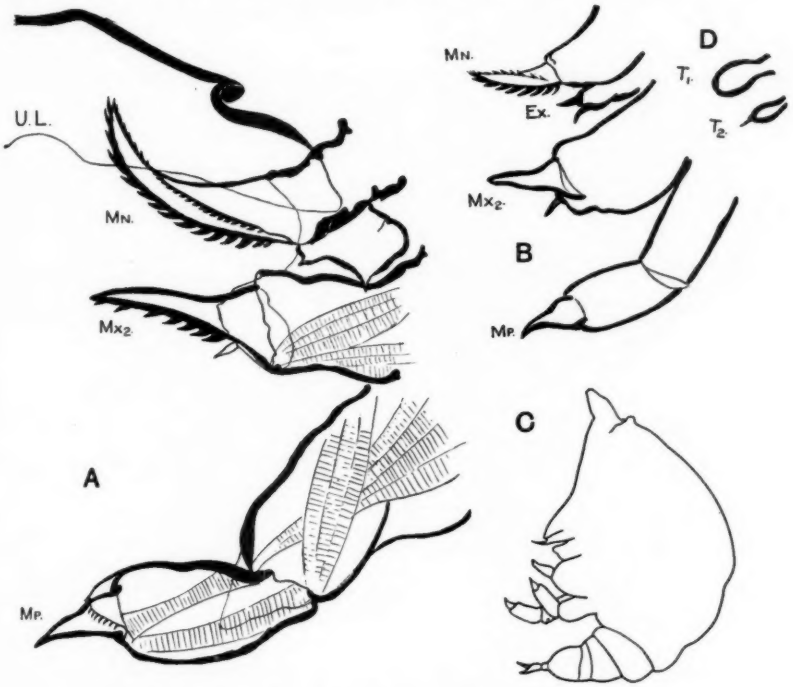

Fig. 2. *Chondracanthus merluccii*. A = mouth-parts of ♀; B = mouth-parts of ♂; C = lateral view of ♂.

Genus **Acanthochondria** Oakley, 1927.

Female. Chondracanthinae with cephalic barbs, dorsal processes and ventral processes absent. Thoracic legs bipartite.

Male. Of intermediate size. ♀ : ♂ = 10 (average). Maxillae toothless. This genus is made up of species transferred from the old genus Chondracanthus, which with *A. exilipes* Wilson (MS. note) gives 17 species.

Acanthochondria cornuta (O. F. Muller) 1777.

Female: appendages (Fig. 3 *A*). Mandibular palp small unilobular; second maxillae with one terminal and ten lateral teeth; maxillipedes long and narrow, cap of spines hooked; basal enlarged into circular plate, terminal blunt. Upper lip triangular, distal edge the wider. Thoracic legs two pairs biramous.

Male (Fig. 3 *B*). Cephalothorax inflated, though not to a marked degree; second thoracic segment largest of free ones.

Appendages (Figs. 3 *C* and *D*). First antennae large, unjointed; second maxillae with small basal spine; maxillipedes long and slender, terminal article long and hooked. Thoracic legs laminate, each with one terminal spine.

Parasitic in the gill covers of *Pleuronectes platessa*.

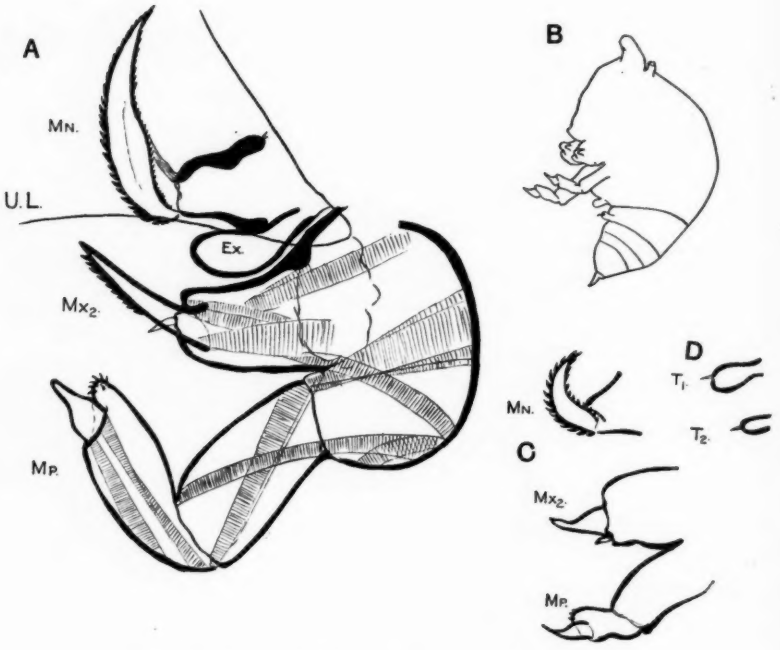

Fig. 3. *Acanthochondria cornuta*. A = mouth-parts of ♀; B = lateral view of ♂; C = mouth-parts of ♂; D = thoracic legs of ♂.

Acanthochondria clavata (Bassett-Smith, 1896).

Female: appendages (Fig. 4 *A*). Mandibles large; mandibular palp large, indistinctly bilobed, with medial and posterior spines; second maxillae with inflated basal article, one terminal and eight lateral teeth; basal spine very short; maxillipedes weak.

Male (Fig. 4 *C*). Cephalothorax inflated, with slight anterior pointing; second, third and fourth segments much the same size, somewhat inflated.

Appendages (Fig. 4 *D*). Mandibular teeth large, palp of average size, with one terminal seta; second maxillae with terminal article a broad tapering lamina; maxillipedes stouter than usual. Thoracic legs small laminae.

Parasitic on the gills of *Pleuronectes microcephalus*.

Acanthochondria depressa (T. Scott, 1905).

Female: Appendages (Fig. 4 *B*). First antennae short, somewhat inflated; mandibular palp large with medial and posterior spines; second maxillae with large basal article, not inflated, terminal with seven large teeth; basal spine unusually stout and strong. Maxillipedes strong, terminal article a mammillated claw.

Male (Fig. 4 *D*). Cephalothorax considerably inflated, with suggestion of anterior pointing, antennae borne at the very tip; second, third and fourth segments much the same size, abdomen very small.

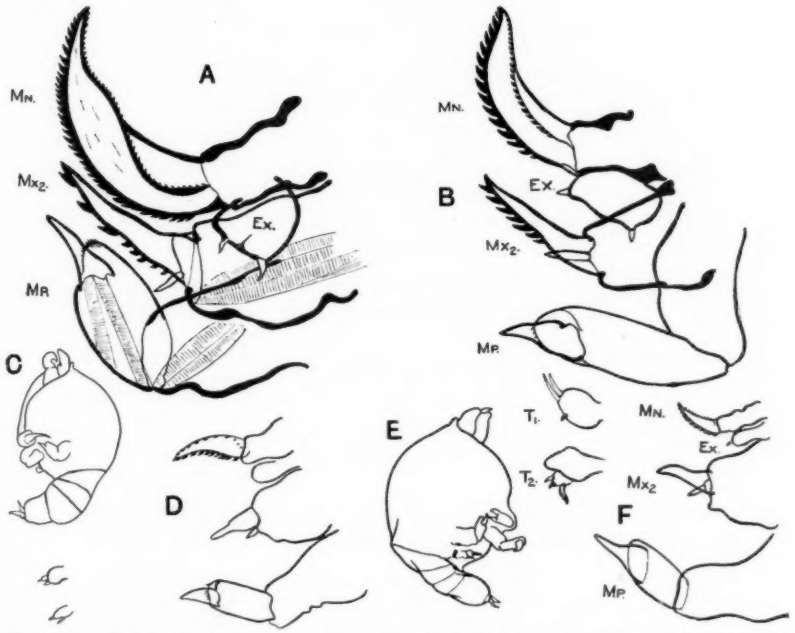

Fig. 4. *Acanthochondria.* A = *A. clavata,* mouth-parts of ♀; B = *A. depressa,* mouth-parts of ♀; C = *A. clavata,* lateral view of ♂; D = mouth-parts of ♂; E = *A. depressa,* lateral view of ♂; F = mouth-parts of ♂.

Appendages (Fig. 4 *F*). First antennae indistinctly three articled; mandibular palp shorter than basal article of mandible, with single terminal seta; second maxillae with large non-inflated basal, basal spine stout but small; maxillipedes slender with basal enlarged at point of attachment. Thoracic legs somewhat elongated laminae, with small postero-terminal spines.

Parasitic on the gills of *Pleuronectes flesus.*

Remarks. T. and A. Scott (1912–13) state that "no males have been observed." Examination of their specimens in the British Museum Collections (1913.9.18.354–63) shows that almost all the females bear males. From several

of these, which are unfortunately in a poor state of preservation, the above account of the male is derived.

Acanthochondria epachthes (Wilson, 1908).

Remarks. The one point of interest in this species is the host, *Hydrolagus* [*Chimaera*] *colliei*, a member of the Holocephali. Some points in its anatomy rather suggest the genus *Acanthochondrites*, but as Wilson neither describes nor figures the swimming legs, this is rendered improbable, as such a careful observer would scarcely have failed to remark on such a striking difference.

Acanthochondria flurae (Krøyer, 1863).

Female: appendages (Fig. 5 *G*). Second antennae unusually large, bent almost at a right-angle; mandibles small, teeth large; second maxillae with one terminal and ten lateral teeth; maxillipedes long but weak.

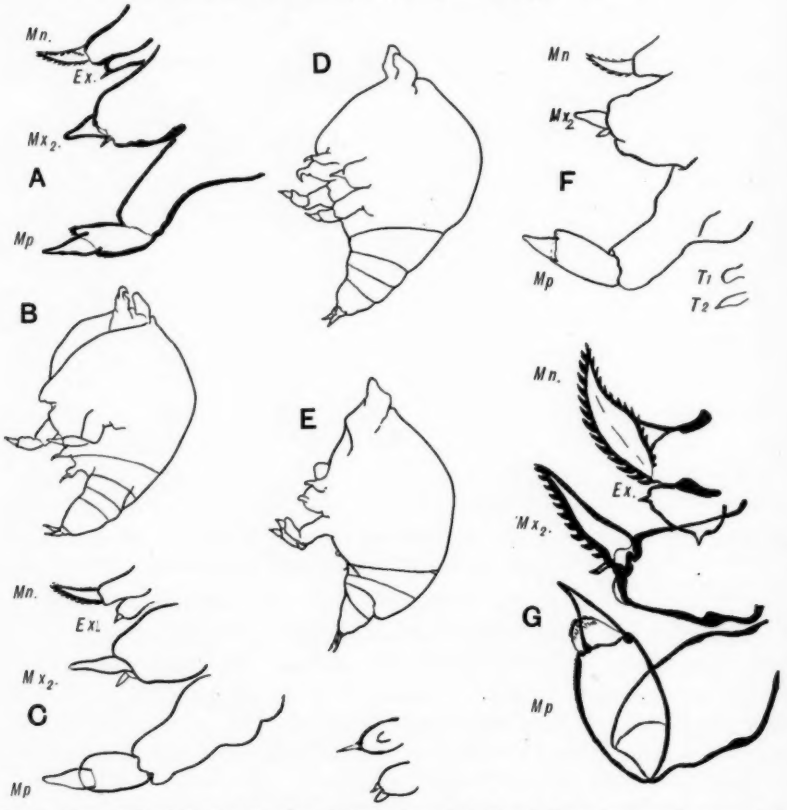

Fig. 5. *Acanthochondria*. A = *A. soleae*, lateral view of ♂; B = *A. limandae*, mouth-parts of ♂; C = *A. soleae*, mouth-parts of ♂; D = *A. limandae*, lateral view of ♂; E = *A. flurae*, lateral view of ♂; F = mouth-parts of ♂; G = mouth-parts of ♀.

Male (Fig. 5 *E*). Cephalothorax inflated, mouth-parts more anterior than usual, anterior end pointed; free thorax segments all same size; animal very much less curved on itself than usual.

Appendages (Fig. 5 *F*). Mandibles elongated, mandibular palp large, spineless; second maxillae with square inflated basal; maxillipedes very slender. Thoracic legs: first pair circular laminae, with two posterior spines; second pair more elongated, one medial and one posterior spine.

Parasitic on the gills of *Drepanopsetta platessoides*.

Acanthochondria limandae (Krøyer, 1863).

Female. The tubercles on the dorsal surface of the second thoracic segment are probably homologous with the dorsal processes of the Lernentominae and the genus *Chondracanthus*. No females available for further description.

Male (Fig. 5 *D*). Cephalothorax inflated, not pointed anteriorly; second thoracic segment largest, third and fourth equal in size; abdomen larger than usual, abdominal appendages short.

Appendages (Fig. 5 *B*). Mandibles long and slender, mandibular palp slightly shorter than basal article, one terminal spine. Second maxillae with short terminal article; maxillipedes long and slender. Thoracic legs laminate, with two and one terminal spines. Male not much curved on itself.

Parasitic on the gills of *Pleuronectes limanda*; rare.

Acanthochondria purpurea n.sp.

Material. 7 ♀♀, 5 bearing each 1 ♂, from *Eleginops maclovinus* (British Museum Collection, 1920.7.5.73–7). Collected by A. G. Bennett, presented by the Governor of the Falkland Islands.

Locality: S. Orkneys.

Female (Fig. 6 *A*). Head indistinctly separated from first thoracic segment, bluntly triangular, length equal to breadth, antennae at tip, slightly separated from the mouth-parts, which are buried in a pit formed by the upper lip anteriorly and the "cheeks" laterally. First thoracic segment small, half as long as the head, same width; second thoracic segment nearly length of head. Remaining thoracic segments fused to form a genital segment, deeply constricted about halfway along its length; dorsally the constriction is transverse, ventrally convex anteriorly. Genital segment one and a half times length of head and pregenital thorax, oval in shape; posterior processes stout and thick, like those of *Chondracanthus lophii*; in the triangular bay between them is the median posterior prolongation of the genital segment, and the small, spherical, unarmed, unsegmented abdomen. Egg-strings tapering, equal in length to body, pointing dorsally.

Length. 10·5 mm.

Colour. Body deep purplish-brown, egg-strings orange.

Appendages (Fig. 6 *B*). First antennae with basal article inflated, terminal cylindrical, with few setae; mandibles with basal and terminal articles equal

in length, mandibular palps equal in length to basal article, no setae; second maxillae large, terminal article bearing one terminal and thirteen lateral teeth; maxillipedes rather weak. Thoracic legs two pairs, indistinctly biramous. Upper lip a triangle, attached by its base, and split down the middle line.

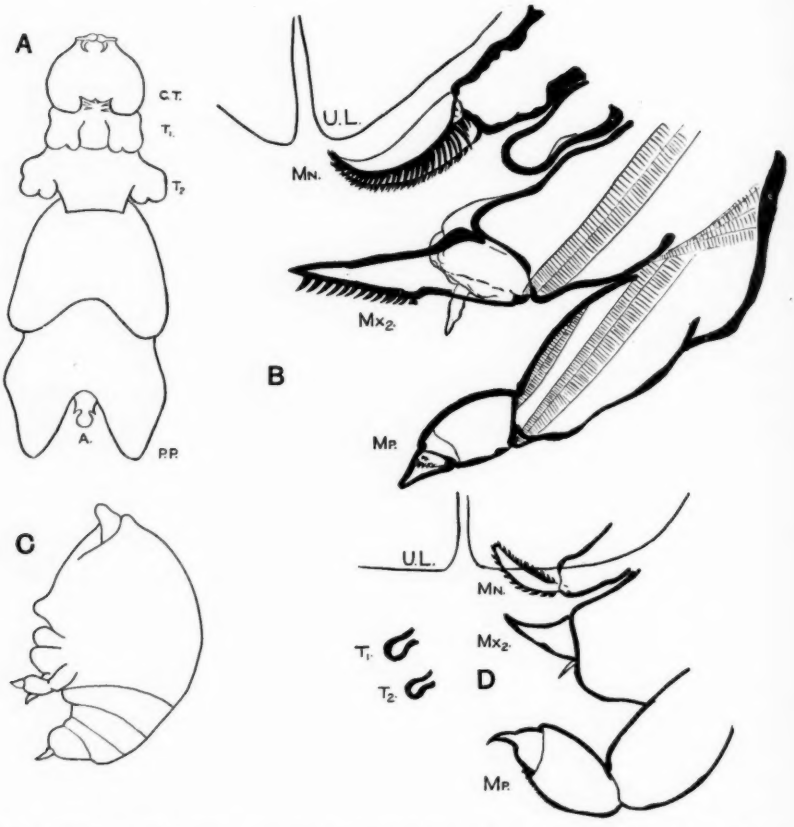

Fig. 6. *Acanthochondria purpurea* n.sp. A = ventral view of ♀; B = mouth-parts of ♀; C = lateral view of ♂; D = mouth-parts of ♂. *C.T.* = cephalothorax; *P.P.* = posterior process; *A.* = abdomen.

Male (Fig. 6 *C*). Much more robust than usual, generally inflated; second, third and fourth thoracic segments about equal in size.

Appendages (Fig. 6 *D*). First antennae cylindrical, three articled; mandibles with two articles equal in length, teeth large; second maxillae with basal article very much inflated, terminal article as long as basal, short basal spine. Maxillipedes fairly stout, terminal article a mammillated claw. Thoracic legs two pairs, small, non-setose laminae.

Remarks. It is stated on the label that this species is an external parasite of *Eleginops maclovinus*, which is probably to be interpreted as meaning either (1) parasitic on the operculum rather than on the gills, or (2) attached to, but not buried in, its host. It may very readily be recognised by its deep purplish-brown colour and bright orange egg-strings. The male is distinguished by its unusually robust character.

Acanthochondria soleae (Krøyer, 1838).

No **females** available for description.

Male (Fig. 5 *A*). Cephalothorax inflated, no anterior pointing; second thoracic segment largest, third and fourth about same size.

Appendages (Fig. 5 *C*). Second antennae very strongly uncinate; mandibles with small teeth, mandibular palp small, with one terminal seta; maxillae inflated; maxillipedes very long and slender. Thoracic legs large laminae, with lateral projections and two terminal setae.

Parasitic on the gills of *Solea vulgaris*: rare.

Remarks. It has been suggested by Brian (1906) that *A. flurae* and *A. soleae* are varieties of *A. cornuta*. I cannot agree with this view for the following reasons: (1) the proportion of the body regions is very different; (2) the appendages show definite differences; (3) the males are easily distinguishable (cf. Figs. 3 *B*, 5 *C*, 5 *E*); (4) the hosts are specific characters.

Acanthochondrites gen. nov.

Female. A large form, similar in general appearance to *Acanthochondria*, but with thoracic legs undivided, unjointed.

Male. Very large. ♀ : ♂ = 5. Cephalothorax enormously inflated, making remaining parts appear ridiculously small. Maxillae toothed along the inner border.

One species: *Acanthochondrites* [*Chondracanthus*] *annulatus* (Olsson, 1868).

Acanthochondrites annulatus (Olsson, 1868).

Male. Cephalothorax enormously inflated; remaining segments very small, and about equal in size, abdominal appendages large.

Appendages (Fig. 8 *A*). Second antennae rather small, and not so strongly uncinate as in other species; mandibles stronger than usual, with fewer but stronger teeth, terminal blade broader than usual. Second maxillae with basal article extremely inflated, much wider than long, with long basal spine; terminal article broad and tapering, armed with four sharp lateral teeth, increasing in size proximo-distally; maxillipedes long and slender. Thoracic legs two pairs, flat, unjointed laminae.

Remarks. This species may be easily recognised by the undivided swimming legs of the female, and the large size of the males.

Genus **Chondracanthopsis** Wilson (MS. note).

Female. Head separated from first thoracic segment, wider than long; first two thoracic segments completely separated, remaining segments completely fused, and provided with a row of knobs down each lateral margin, and a row of transverse laminae down the dorsal mid-line. Thoracic legs two pairs, uniramous, made up of a very wide basal, and a boot-shaped terminal segment.

Male. Head fused with first thoracic segment, pointed anteriorly; not much inflated, bearing antennae at the very tip; maxillae toothed on the inner margin.

Type species: *Chondracanthopsis* [*Chondracanthus*] *nodosus* (Muller, 1777).

One other species: *C. cottunculi* (Rathbun, 1887).

Chondracanthopsis nodosus (Muller, 1777).

Female (Fig. 7 *A*). As in genus diagnosis.

Appendages (Fig. 7 *B*). First antennae unsegmented, basal part inflated, terminal part narrowed down to a knob at the tip; mandibular palps large with medial and posterior spines; second maxillae provided with one terminal and eleven small lateral teeth; maxillipedes with area of attachment enlarged to circular plate. Thoracic legs two pairs, uniramous, two jointed, basal very wide, terminal boot-shaped.

Male (Fig. 7 *C*). As in genus diagnosis.

Appendages (Fig. 7 *D*). Mandibular palp large, with single terminal seta; second maxillae of usual shape, with two small lateral teeth on the long terminal article. Maxillipedes with two proximal articles equal. Thoracic legs each with two apical spines.

Remarks. Perhaps the most interesting character of this genus (not mentioned in the genus diagnosis, as it does not appear to be constant) is the presence of conjunctive tubes, doubtfully associated with males, described by Leigh-Sharpe and Perkins (1924). The same phenomenon occurs also in *Lernentoma asellina* (Linnaeus), where however the association with males is more definite. Here the condition is complicated by the co-existence of epigynic males (as described above), which does not occur in *L. asellina*.

Disphaerocephalus gen. nov.

Female (Fig. 8 *B* and *C*). Head triangular, apex anterior, provided with one pair of large sub-spherical lateral expansions; indistinctly separated from first thoracic segment; no cephalic neck. First thoracic segment bears one pair of small, distinctly biramous legs pointing posteriorly; second thoracic segment bears dorsally one pair of large dorsal processes, and ventrally one pair of legs, clumsy, tripartite legs, pointing anteriorly. Remaining thoracic segments fused to form a genital segment, with large lateral projections, and small convergent posterior processes enclosing an unsegmented abdomen. Three median dorsal lobes. **Males** unknown.

One species: *Disphaerocephalus* [*Chondracanthus*] *ornatus* (T. Scott, 1912).

Remarks. The great interest of this species lies in its obvious relationship to the Lernentominae; the animal looks like an *Oralien* which has not yet

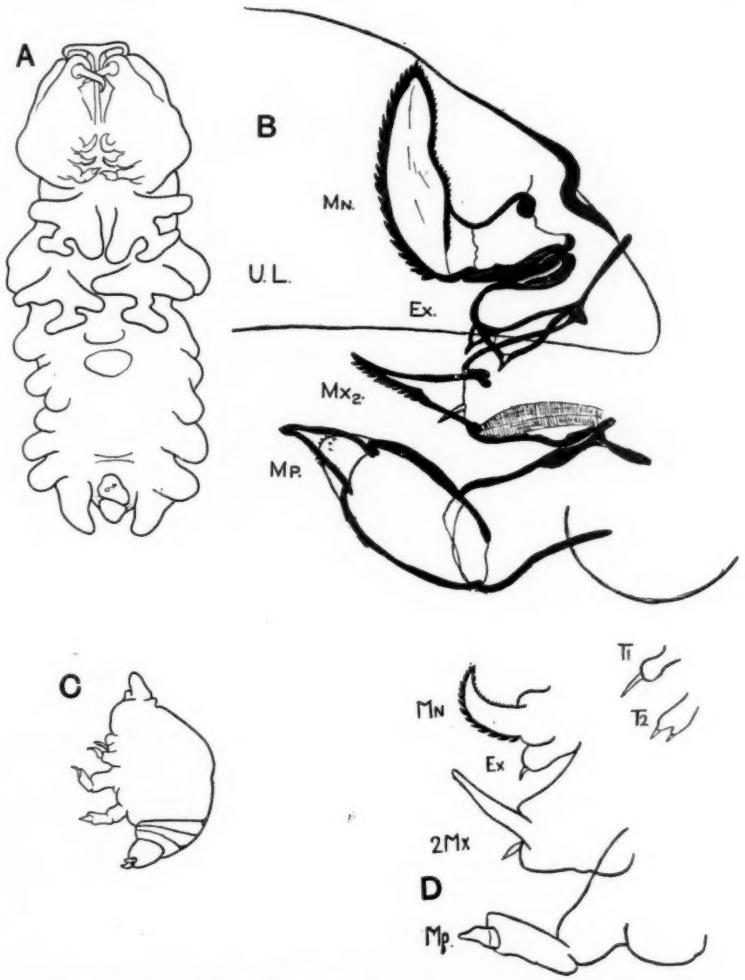

Fig. 7. *Chondracanthopsis nodosus*. A = ventral view of ♀; B = mouth-parts of ♀; C = lateral view of ♂; D = mouth-parts of ♂.

developed a cephalic neck. Dr Wilson, in a MS. note, has suggested that this species be transferred to his new genus *Chondracanthopsis*. While I agree that the published figures wholly bear out his view, inspection of the original specimens in the Collections of the British Museum shows that the present

species differs markedly from *Chondracanthopsis* in the possession of dorsal processes, lateral expansions, and biramous and tripartite legs; so that a new genus has been erected for it. With this view Wilson now agrees.

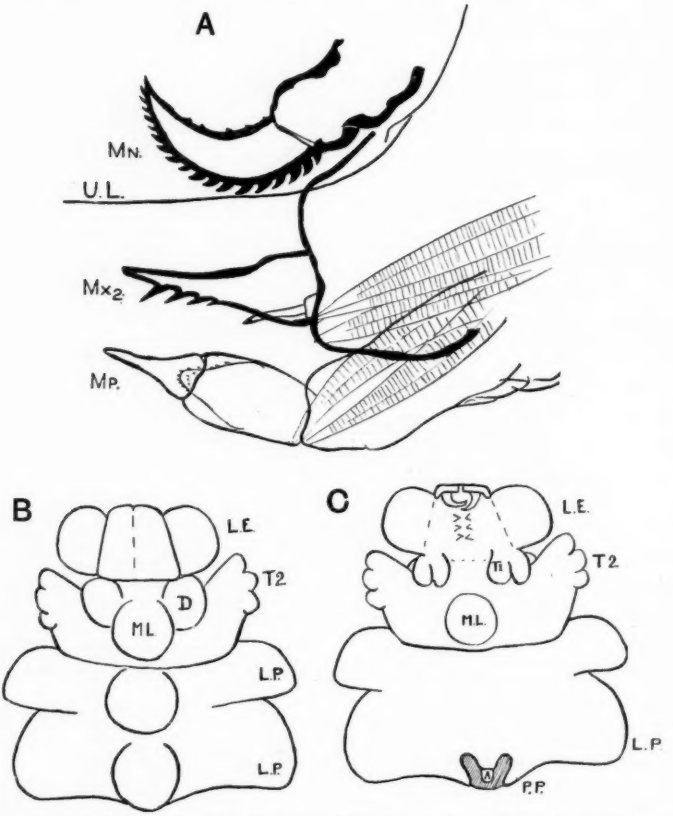

Fig. 8. A = *Acanthochondrites annulatus*, mouth-parts of ♂; B = *Disphaerocephalus ornatus*, dorsal view of ♀; C = ventral view of ♀. *L.E.* = lateral expansions; *L.P.* = lateral processes; *D* = dorsal process; *P.P.* = posterior process; *M.L.* = median lobe.

Genus **Blias** Krøyer, 1863.

Female. Head inflated, separated from thorax by a marked constriction; trunk similarly inflated, no segmentation apparent; thoracic legs two pairs, prehensile.

Male. Like that of *Chondracanthus*; numerous on a single female.

Type species: *Blias prionoti* Krøyer, 1863.

Remarks. From Krøyer's wretched little figures it is impossible to come to any satisfactory conclusion regarding this animal, except that it is a Chondracanthid. The genus diagnosis is strangely equivocal; the "prehensile

limbs" might be either thoracic legs or maxillipedes. I incline to the former view, as Krøyer would hardly emphasise a condition common to all the Chondracanthidae; and if this is so, the creation of a new genus is amply justified.

Genus **Chondracanthodes** Wilson (MS. note).

Female. Head separated from the first thoracic segment, considerably elongated, strongly deflexed; thorax more or less distinctly four jointed; first segment bearing only first legs; second and third segments each with a blunt process on each lateral margin; fourth segment with a similar process on each lateral corner, all the processes turned ventrally. Second segment with a third process on the dorsal mid-line, projecting forwards over first segment; flattened knobs on dorsal surface of third and fourth segments; abdomen two articled, with minute abdominal appendages.

Male. Head fused with first thoracic segment, carapace projecting in a rounded knob on either side opposite the mouth; trunk four segmented; abdominal appendages stout.

Type species: *Chondracanthodes deflexus*, parasitic on the gills of *Macrourus bairdii*.

Locality. Massachusetts coast.

Genus **Diocus** Krøyer, 1863.

Female. Head small, fused with first thoracic segment, strongly deflexed, provided with one pair of small cephalic barbs; second thoracic segment free, provided with one pair of long dorsal processes knobbed at the extremity. Remaining thoracic segments fused to form a genital segment, which is divided into two equal parts by a deep constriction. The anterior half is twice as broad as long, and bears convergent latero-ventral processes, bluntly trifid at the extremity; the posterior half is the mirror image of the anterior, the trifid processes enclosing an unjointed abdomen. Thoracic legs absent. Egg-strings spirally coiled.

Type species: *Diocus gobinus* (Muller, 1777).

Remarks. There is unfortunately only one specimen of this species in the British Museum Collections, so that no details of appendages can be given. No male present.

Host. *Cottus gobio*; common locally.

Genus **Pseudochondracanthus** Wilson, 1908.

Female. Head quadrilateral, indistinctly separated from the first thoracic segment, covered by a marked dorsal carapace. All remaining thoracic segments fused to form an unsegmented genital segment, provided with short, blunt, parallel posterior processes, enclosing a one segmented abdomen. Second maxillae with terminal article a broad flange, toothed on both margins; maxillipedes with enormously inflated basal article, forming cheeks of head; palp large and inflated. Thoracic legs one pair, bluntly biramous at the extremity.

Male. Of intermediate size. ♀ : ♂ = 7. Rather pointed anteriorly, segmentation not very obvious; terminal article of maxillipede a flattened setose lamina. Maxillae toothed along their inner margins. Thoracic legs absent.

One species: *Pseudochondracanthus diceraus* Wilson, 1908.

Triphyllacanthus gen. nov.

Female. Head sub-spherical, separated from inflated trunk by a slight constriction, no definite segmentation. Second antennae tripartite, the digitations blunt and flattened; first pair of thoracic legs bipartite, unjointed; second pair undivided, unjointed, with terminal spine.

One species: *Triphyllacanthus molestus* (Heller, 1865).

The reasons for transferring this species have already been stated.

ACKNOWLEDGMENTS.

I wish to express my gratitude to Dr Chas. B. Wilson of the State Normal School, Westfield, Mass., for his MS. notes on *Chondracanthodes* and *Chondracanthopsis*, and considerable other assistance; to Mr W. H. Leigh-Sharpe for many suggestions; and to Miss E. C. Humphreys for the drawings of the males.

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(*MS. received for publication* 23. IX. 1929.—Ed.)

ON SOME BRITISH LARVAL CESTODES FROM LAND AND FRESH-WATER INVERTEBRATE HOSTS.

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(With 5 Text-figures.)

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I. INTRODUCTION.

There are few records of British larval Cestodes based on a systematic search of the Invertebrate fauna. The descriptions available in the literature relate for the most part to cystic stages (chiefly from Vertebrata) of tapeworms parasitic in man or domestic animals. In this country, however, among those who have contributed towards a knowledge of this group, relevant to the present theme, must be mentioned the name of Rosseter (1890, 1894, 1897). The considerable record of Continental larval Cestodes is due to the work of von Linstow (1875, 1892 *a*, *b*), Mrazek (1890, 1891; 1907, 1916) and Dady (1901).

The larval Cestodes described in the present paper were obtained in the course of a systematic search of the land and fresh-water Invertebrata of the Dundee and Arbroath districts for larval helminths. The material collected

was investigated by methods similar to those described in a former paper (Harper, 1929). Experiments for the further elucidation of life-histories, using ducklings as final hosts, were performed under a vivisection licence from the Home Office. The work was carried out in the Department of Natural History, University College, Dundee, under the late Prof. Gemmill, F.R.S., during my tenure of a Research Maintenance Allowance from the Department of Scientific and Industrial Research (1923–5) and a Carnegie Research Scholarship (1925–6).

The larvae described are arranged according to Lühe's classification (1910, p. 132), in which the primary grouping is based mainly on the character of the caudal appendage. Five of the ten species brought together in this paper I have related to their corresponding adults, while in the absence of further evidence as regards their life-histories, I have referred to the others as *Cysticercus "A," "B,"* etc.

II. DESCRIPTIONS OF PARASITES.

GROUP I. (LÜHE, 1910, pp. 132–42.)

("Cysticercoide mit langem unverästeltem Schwanzanhang (*Cercocystis* Vill.).")

These tailed forms develop for the most part in Entomostraca, while the adults in known instances occur in water-fowl. The body varies in form from spherical to flattened, and in contour from circular to oval. The wall, as a rule, is thin, but may in some cases, *e.g. Cysticercus Hymenolepidis setigerae*, possess a distinct gelatinous outer covering (cf. Fig. 2 *B*). Calcareous bodies are often present, particularly in the future "neck" of the larva. The six hooks of the hexacanth embryo are usually discernible in the tail.

In all cases examined by me the cysts projected into the hollow of the host's body with the tail surrounding the intestine and securely anchored in the musculature. The latter observations agree with those of Lindner (1921) for *Cysticercus Hymenolepidis gracilis*, from the Ostracods *Dolerocypris fasciata* Müll., and *Candona neglecta* Sars (var. *tuberculata*). The mode of attachment of the tail within the body of the host is not definitely known. Slight variations sometimes occur in the form and size of the same species of cysticercus, a feature most commonly met with in cases where the intermediate host is not specific. I have observed this, for example, in *Cysticercus "A"* (p. 205, Fig. 1 *B*), a new species which occurs locally in the Ostracods *Eurycypris pubera* and *Cypris reptans*. Lindner (*loc. cit.* p. 38) thinks these modifications, especially in Ostracod cysts, may be due in part to the nature of the body cavity and the osmotic pressure of the body fluids of the different intermediate hosts.

Further development. The early workers considered that the relation between larva and adult might be inferred from careful comparison and measurement of the hooks of the scolex, a view which was strongly upheld by Rosseter in this country. "I have long held the opinion," said that worker (1897, p. 398), "that the hooks of the scolex, so variably constituted as they

are in form and size in each species of tapeworm, should play an important part as a distinctive feature in classifying the armed Taenia in the cysticercoid stage." Conclusive proof of the life-cycle, however, can only be obtained by experimental infection, either of final hosts, using known larval stages to obtain the adult, or of intermediate hosts, using the eggs of known tapeworms to obtain the corresponding larval stage. It was by the first of these methods, namely by feeding likely final hosts with known larval stages, that I have been able to give information regarding the further development of some of the local forms. Here reference must be made to the feeding experiments performed by Rosseter (1891, 1892, 1893, 1897) whose results show, incidentally, how accurate were some of the conclusions drawn by the early workers concerning relations based on a careful examination of the scolex and its armature.

The life-history of Hymenolepis tenerrima *v. Linst.* (Fig. 1 *A*.)

The larval stage was obtained from the Ostracod *Cypris reptans*. The cyst is transparent and almost spherical. Its average dimensions are: length 0·33 mm., breadth 0·29 mm. A thin gelatinous zone surrounds the cyst. The tail measures from three to four times the length of the body, and four of the embryonic hooks (length 0·006 mm.) were observed near its distal end. There are four oval suckers measuring 0·106 × 0·06 mm., and the rostellum carries ten relatively large recurved hooks 0·11 mm. in length, with their apices set close together. Calcareous granules are fairly abundant.

During April 1926 approximately 10 per cent. of the Ostracods examined from Morton Loch, Fifeshire, were infected, each infected host invariably containing two cysts.

Experimental. In April 1926 eight larvae freshly isolated from their hosts were introduced into the intestine of a duckling by means of a pipette. The duckling, as obtaining in the other feeding experiments recorded in this paper, was hatched and reared in the Department. It was fed on one occasion only and killed 20 days later, when two immature specimens of *Hymenolepis tenerrima* (v. Linst. 1882) were recovered from the large intestine. This tapeworm occurs naturally in *Nyroca fuligula* L. and *Nyroca marila* L. Under the conditions obtaining in the experiment it would appear that the worm is unable to reach sexual maturity.

As far as I am aware no corresponding larva has been described for *Hymenolepis tenerrima* v. Linst.

Cysticercus "A." (Fig. 1 *B*.)

This larva occurs locally in the Ostracods *Eurycypris pubera* and *Cypris reptans*. It is greyish-white in colour and varies in shape from broadly oval to almost spherical. The measurements are: length 0·3–0·34 mm., breadth 0·25–0·28 mm. The larva is surrounded by a clear zone, approximately 0·01 mm. in thickness. The tail is exceedingly translucent and measures from 1·2–1·6 mm. in length. Four embryonic hooks (length 0·007 mm.) are present near its distal end, and the other two occur about 0·3 mm. from the base of

the cyst. The suckers are oval in outline, measuring 0·08 × 0·06 mm., and their margins are spiny. The rostellum is armed with ten hooks, 0·077 mm. long. Calcareous granules are present but are not numerous.

This cysticercus occurs most abundantly in *Eurycypris pubera*, and during October 1925 was present in between 40 and 50 per cent. of the specimens dissected. During the same month it occurred in about 10 per cent. of *Cypris reptans*. In all cases only one larva was found in any one host.

Cysticercus "A" bears some resemblance to *Cysticercus Hymenolepidis anatinae* from the Ostracods *Cypria ophthalmica* Jur., *Cypris incongruens* Ramd. and *Cypris ovata* (Mrazek, 1891, p. 128; 1916, pp. 532–5; Moniez, 1891;

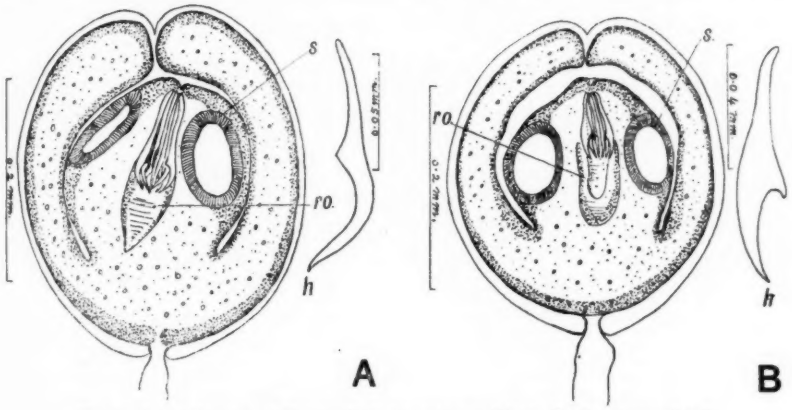

Fig. 1. A. Cysticercus of *Hymenolepis tenerrima* v. Linst. B. *Cysticercus "A."* *h.* hook of rostellum; *ro.* rostellum; *s.* sucker.

Lühe, 1910, pp. 138–9 and 73–4). The result of the infection experiments recorded below, however, show that the two cannot be identical.

Experimental. As a result of controlled feeding experiments, using ducklings as final hosts, I obtained a number of sexually immature tapeworms. Of these Dr Baylis of the British Museum says: "...the species is likely to be new. It does not agree with any described species of *Aploparaksis* but apparently belongs to that genus as I can only make out one testis per segment. The suckers are spiny as in some species of *Diorchis*, but in this genus there are two testes."

In this case, therefore, both larva and adult are new, but so far I have been unable to obtain adult material sufficient for description.

Cysticercus "B." (Fig. 2 *A*.)

This cysticercus, which occurs in *Eurycypris pubera*, is transparent and oval. The long diameter is 0·33 mm. and the median diameter 0·23 mm. A long and very transparent caudal appendage is present, measuring in some cases as much as 2·6 mm. long, that is, roughly eight times the

length of the body. The six hooks of the hexacanth embryo can be observed in the tail; four in two groups of two each, near the distal end, and another group of two approximately one-sixth the distance from the end. These hooks measure 0·006 mm. in length. The four suckers stand out boldly in the living specimen, their margins being tinged with brown pigment. They are 0·08 mm. in diameter and covered with numerous transverse rows of minute spines. The rostellum is armed with ten hooks measuring 0·03 mm. in total length and they are commonly found inclined at an angle as shown in the figure. Calcareous granules are numerous in the future "neck."

During September and October 1924 this larva occurred in about 5 per cent. of the host species examined from Morton Loch, Fifeshire, each infected Ostracod containing one cyst.

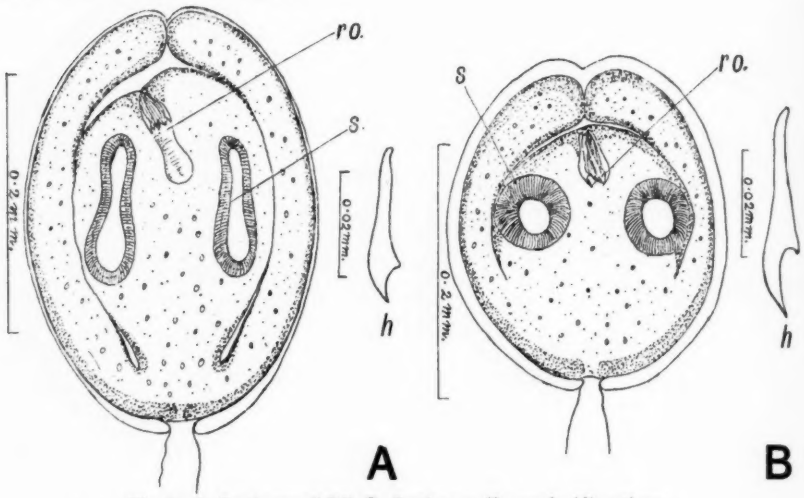

Fig. 2. A. *Cysticercus "B."* B. *Cysticercus Hymenolepidis setigerae.*
(For lettering see Fig. 1.)

There appears to be no cysticercus described in the literature with hooks similar to the above species. The hooks resemble those of *Drepanidotaenia lanceolata* Bloch, but in that species there are only eight hooks.

Cysticercus Hymenolepidis setigerae *from a new host.* (Fig. 2 *B.*)

Cysticercus Hymenolepidis setigerae (v. Linstow, 1892 *b*) is found locally in the Ostracod *Eurycypris pubera*. It is transparent and slightly longer than broad, the measurements being: length 0·24 mm., and the breadth 0·2 mm. A gelatinous zone 0·02 mm. in thickness surrounds the larva. The tail is relatively long, measuring from 1·5 to 2·0 mm., and four of the embryonic hooks were observed some distance from its distal end. The suckers are circular in outline with a diameter of 0·06 mm. The rostellum is active and carries ten hooks, 0·042 mm. in length. Calcareous granules are not abundant.

During September 1924 approximately 10 per cent. of the host species were infected, each infected specimen containing one larva.

Cysticercus Hymenolepidis setigerae has previously been recorded from *Cyclops strenuus* Fisch.; *Cyclops* sp., and *Diaptomus* sp. I have seen no record of its occurrence in Ostracods. Its hooks resemble those of the adult *Hymenolepis setigera* Frölich, which occurs naturally in the intestine of various wild ducks and swans.

GROUP II. (LÜHE, 1910, p. 142.)

("*Cysticercoide* mit verästeltem Schwanzanhang.")

The life-history of Aploparaksis dujardinii *Krabbe.* (Fig. 3 *A*.)

The cysticercus of *Aploparaksis dujardinii* Krabbe occurs in the body cavity of the terrestrial Oligochaetes *Fridericia ratzeli* Eisen and *Mesenchytraeus harperi* sp. n. (Stephenson, 1926).

The cyst is semi-transparent and varies from oval to almost circular in outline. Its average measurements are: length 0·36 mm., and breadth 0·25 mm. The much-branched and very transparent tail may exceed twice the length of the cyst itself. The branches are of variable length and take origin from a main caudal stem. They are as a rule simple, but may give rise to smaller secondary branches or lobes. I have been able to detect only two of the embryonic hooklets; they occurred near the tip of the main caudal stem and measured 0·007 mm. in length.

Usually, the parasite is located posterior to the centre of the host's body. The cyst itself projects into the coelom and is moored to the intestine and body wall by means of its ramifying tail. The four suckers (diameter 0·08 mm.) are spiny, and tinged with brown pigment on their margins. The hooks on the rostellum are 46 in number and are arranged in a complete circlet. They measure 0·02 mm. long by 0·17 mm. broad. Calcareous granules are abundant.

During November and December 1924 the infection of both host species ranged from 12–15 per cent. As a rule only one parasite is present in one host, although I found as many as ten.

A search of the intestines of wild birds in the locality in which the infected Oligochaetes occur provided a number of adult Cestodes with hooks corresponding in every respect to those of the cysticercus described above. These adults were obtained from *Turdus iliacus* and *T. merula*. Dr Baylis has determined the species as *Aploparaksis dujardinii* (Krabbe, 1869) which has previously been recorded from *Turdus musicus*, *T. iliacus* and *Sturnus vulgaris*. As far as I am aware no larval stage has been described for the species.

GROUP III. (LÜHE, 1910, p. 143.)

("*Cysticercoide*, deren Schwanzteil die 'Cyste' völlig umwachsen hat.")

The life-history of Aploparaksis furcigera *Nitzsch.* (Fig. 3 *B*.)

It would appear that only one European species belonging to this group has been described, namely *Cysticercus Aploparaksidis crassirostris* from *Lumbriculus variegatus* Müll. The new form described below was also obtained

from the same species of fresh-water Oligochaete, and by means of direct animal experiment I have shown it to be the larva of *Aploparaksis furcigera* Nitzsch.

The cysticercus is roughly spherical and of a greyish-white colour. It occurs free in the coelom of the host, *Lumbriculus variegatus* Müll., and may be seen shining through the body wall with the unaided eye. As a rule two to four larvae are present within one host, and they are generally located in the middle third of the body. On one occasion 21 larvae were found in a single host. In life the cyst is quite opaque, but with slight pressure under a cover-glass it is seen to consist of a backwardly drawn scolex surrounded by two integuments. Three zones may thus be differentiated: viz. a straw-coloured gelatinous outer zone (= outer integument); a light brown middle

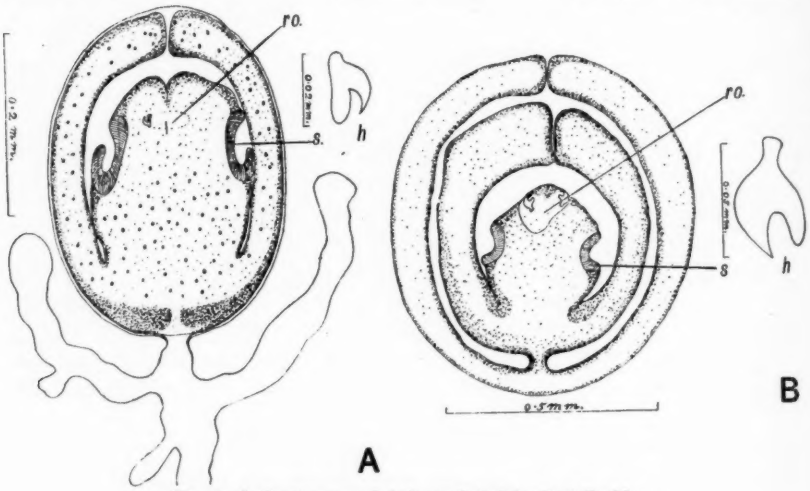

Fig. 3. A. Cysticercus of *Aploparaksis dujardinii* Krabbe.
B. Cysticercus of *Aploparaksis furcigera* Nitzsch.
(For lettering see Fig. 1.)

zone (= inner integument), and a greyish-white central zone (= scolex). The rostellum is armed with four cup-shaped suckers and ten stout recurved hooks. Calcareous granules are present in the future "neck." The length, including the outer integument, is 0·72 mm. and the breadth 0·66 mm. Excluding the outer integument the length is 0·55 mm. and the breadth 0·5 mm. The diameter of the suckers is 0·08 mm. and the hooks measure 0·058 × 0·046 mm.

From October to December 1924, 25–30 per cent. of the host specimens dissected were infected.

It will be observed that the internal anatomy of this larva is very similar to that of *Cysticercus Aploparaksidis crassirostris*. The hooks of the latter, which resemble those of the adult Cestode, *Aploparaksis crassirostris* Krabbe, are however smaller than in the above, being only 0·033 mm. long. The

anterior opening, which Mrazek (1907, Fig. 1, Taf. 30) was the first to demonstrate in the outer integument of *Cysticercus Aploparaksidis crassirostris*, is also present in the larva of *Aploparaksis furcigera* Nitzsch, but in the older larvae of the last-named species the folds which grow up to form this covering appear to fuse completely over the anterior region. It might be noted that Ratzel (1866) records no such anterior opening in what appears to be *Cysticercus Aploparaksidis crassirostris*. Mrazek (1916) describes the development of *Cysticercus Aploparaksidis crassirostris* in detail.

Experimental. A plentiful supply of larvae being available, I was able, during March and April 1926, to conduct a number of controlled feeding experiments, using ducklings as final hosts. As a result, a number of the corresponding adults and a series of intermediate forms from the cysticercus onwards were obtained. The adults were identified by Dr Baylis as *Aploparaksis furcigera* Nitzsch. Infection takes place readily and development is normal. Eggs were found in the faeces 18 days after infection. The habitat is that portion of the intestine just anterior to the rectal caeca.

Aploparaksis furcigera Nitzsch occurs naturally in *Anas boschas* L., *Anas crecca* L., and *Nyroca ferina* L. Its larval stage was previously unknown.

GROUP IV. (LÜHE, 1910, p. 144.)

("Schwanzlose Cysticercoide, welche frei in einer geschlossenen Cyste liegen (*Monocercus* Vill.).")

The life-history of Echinocotyle nitidulans *Krabbe.* (Fig. 4 *B.*)

The cysticercus occurs in the liver of the fresh-water mollusc *Limnaea peregra* Müll. It is dark brown in colour and very small, measuring only 0·19 × 0·15 mm. Anteriorly it is blunt in form and posteriorly rounded. The outer wall is thick and tough and exceedingly resistant to stain. There are four cup-shaped suckers of diameter 0·06 mm. and ten slender hooks measuring 0·056 mm. in total length.

Many Limnaeas have now been dissected, but so far I have found only two infected specimens, one containing one larva and the other, two.

The hooks of this species agree in form and size with those of the adult Cestode *Echinocotyle nitidulans* Krabbe which occurs in the intestine of *Tringa alpina* L. and *Charadrius hiaticula* L., and so far as I am aware no corresponding larva has previously been recorded.

Cysticercus limnaei Villot (1883) is the only other cysticercus described from *Limnaea peregra* Müll. Its hooks resemble those of the adult *Hymenolepis microsoma* Crepl., and are quite different from those of *Echinocotyle nitidulans* Krabbe. It may be noted here that Rosseter (1893) has described a tailed cysticercus from *Cyclops serrulatus* Fisch. as the larva of *H. microsoma*.

Cysticercus "C." (Fig. 4 *A.*)

Cysticercus "C" was obtained from *Lumbriculus variegatus* Müll. It is spherical, with a diameter of 0·49 mm. Only one larva has been obtained so

far and it was located in the brown coloured cells surrounding the alimentary canal of the host. The larval wall is 0·11 mm. in thickness and of a pale yellow tinge. There are 4 suckers of diameter 0·06 mm. and 22 hooks. The latter are arranged in a double row, and, as far as can be determined from the unruptured cyst, the hooks in the two rows measure 0·05 and 0·043 mm. respectively. Calcareous granules are numerous.

Cysticercus "D" is apparently a new larval *Anomotaenia*, for I have seen no similar form described in the literature. Structurally it resembles *Cysticercus Anomotaenia pyriformis* (Mrazek, 1907, pp. 613–14, Figs. 13–16) in which species there are 28 hooks.

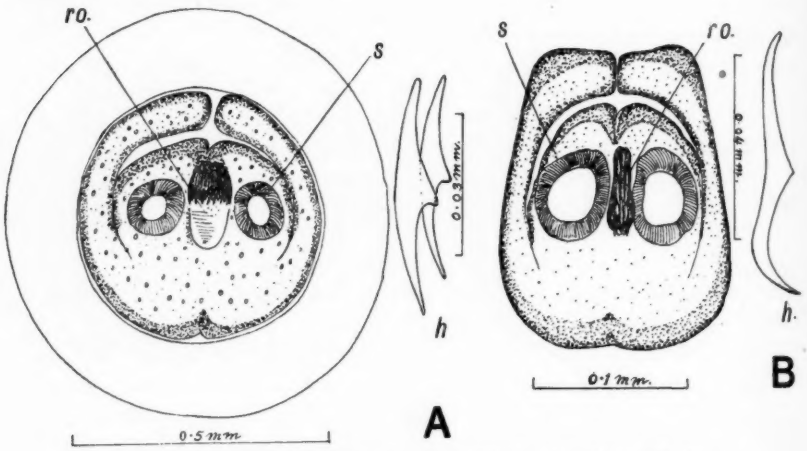

Fig. 4. A. *Cysticercus "C."* B. Cysticercus of *Echinocotyle nitidulans* Krabbe. (For lettering see Fig. 1.)

Cysticercus "D." (Fig. 5 *A.*)

The host species is again *Lumbriculus variegatus* Müll. The central area of the larva containing the scolex is firm and is surrounded by a conspicuous broad orange-yellow zone which widens readily under pressure. The site is the dark brown cellular area around the intestine of the host and from one to six specimens may occur in one Oligochaete. Including the outer zone the length is 0·33 mm. and the breadth 0·31 mm., while excluding it the measurements are 0·24 and 0·20 mm. respectively. No embryonic hooks were observed. The rostellum is long and armed with ten slender hooks of length 0·07 mm. The four suckers are oval in outline and measure 0·11 × 0·08 mm.

During April and May 1925 approximately one-third of the Oligochaetes examined were infected.

Cysticercus "D" is almost certainly identical with an unnamed form described by Mrazek (1907, pp. 614–20, Figs. 17–19) from the same host. The number and size of the hooks do not appear to correspond with those of any

described species of tapeworm. My attempts to secure the adult tapeworm by feeding the larvae to ducklings have proved unsuccessful.

Cysticercus "E." (Fig. 5 *B.*)

This larva was found most abundantly in the terrestrial mollusc *Polita alliaria* Miller, but I have also obtained it to a less extent in *Polita lucida* Drap., *Polita nitidula* Drap. and *Vitrina pellucida* Müll. It is of a dark straw colour and in shape almost spherical. Entirely surrounding it is a clear gelatinous zone approximately 0·056 mm. in thickness. The dimensions including this outer zone are, length 0·50 mm. and breadth 0·48 mm. The larvae are located in the liver of the host, usually in close proximity to the alimentary canal. One to four larvae commonly occur in an infected mollusc, but on one

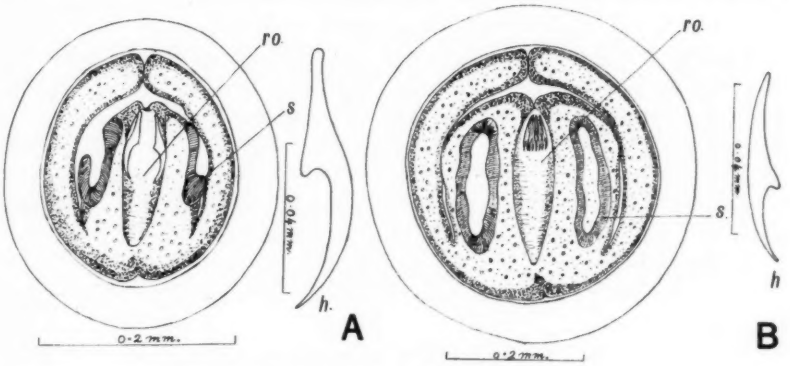

Fig. 5. A. *Cysticercus "D."* B. *Cysticercus "E."*
(For lettering see Fig. 1.)

occasion 22 were found in one host (*P. alliaria* Miller), in which case considerable fusion of the outer walls of the larvae had taken place. The four suckers are oval in outline, measuring 0·2 × 0·08 mm., and their margins are tinged reddish-brown. The long rostellum is armed with 15 slender hooks 0·049 mm. in length. Calcareous granules are abundant and movement of the scolex in the older larvae is pronounced.

During March 1925 over 10 per cent. of the specimens of *P. alliaria* Miller were infected.

I have seen no similar cysticercus described in the literature. Feeding experiments to ducklings have proved negative.

III. SUMMARY AND CONCLUSIONS.

Ten larval Cestodes, eight from aquatic and two from terrestrial hosts, have been described. Of these, eight species are new, and in the case of *Cysticercus "A,"* both larva and adult are new. By direct animal experiment the life-histories of three forms have been definitely established, while by

careful comparison of the hooks of the scolex two larvae have been related to known adults. *Cysticercus Hymenolepidis setigerae*, previously recorded only from Copepods, is described here from an Ostracod host.

Effect of the parasite on the host. It is difficult to estimate the effect of Cestode larvae parasitic in Invertebrata, especially in the smaller forms such as the Entomostraca. In the latter, I have found that the intestine and musculature suffer most, and that the reproductive organs remain unattacked. The same remarks also hold for Oligochaete hosts. Dady (1901) considers that the influence of these cystic stages on their hosts is considerable, particularly on the musculature, sexual organs and intestine, and that death of the host may follow. Lindner (1921) states that little or no harm is done to Ostracods by the presence of tapeworm larvae, and also that the ovary of the female remains unattacked. The same author considers that, among the Entomostraca, destruction of the intestine occurs only in Copepods, the body cavity of Ostracods being so roomy that a harming of the intestine by growth of the parasite is unlikely. Schmidt (1894) finds that for *Cysticercus Hymenolepidis anatinae*, the host, *Cypris ovata*, was not particularly affected.

IV. ACKNOWLEDGMENTS.

I desire to express my thanks to the following authorities through whose kindness the Invertebrate hosts and adult Cestodes were named:

Molluscs: by Mr H. Coates, F.S.A. (Scot.), of Perth.

Crustaceans: by Dr W. T. Calman, F.R.S., of the British Museum (Natural History).

Oligochaetes: by Dr J. Stephenson of Edinburgh University.

Adult Cestodes: by Dr H. A. Baylis of the British Museum (Natural History).

I am also indebted to Prof. A. D. Peacock, D.Sc., F.R.S.E., for advice, and for the use of his laboratory during the preparation of this paper.

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(*MS. received for publication* 21. IX. 1929.—Ed.)

ON THREE NEW CESTODES FROM BIRDS.

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(With 16 Text-figures.)

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I. *Paraschistometra macqueeni* gen. et sp. nov. from Macqueen's Bustard.

(Text-figures 1–7.)

Of this Cestode I possess about a dozen adult specimens from one bird and nearly two dozen young specimens from another bird, preserved in the collection of the Wellcome Bureau and originally obtained, I believe, by Dr L. Sambon from the London Zoological Gardens. It is probably allied to, though certainly not identical with, the *Ascometra vestita* of Cholodkovsky (1912) obtained from the same host, viz. *Houbara macqueeni*. I have no information as to the locality from which the hosts were obtained in the present case. I shall describe the anatomy of this worm prior to discussing its systematic position.

Anatomy.

The maximum length of the worms attained in my collection is about 110 mm. and the maximum breadth (usually situated at about one-third of the strobila length from the end, but sometimes midway and occasionally even anterior to this) about 6 mm. The proglottids are very numerous and are very variable, both as regards number and individual length. The proglottids in the region of maximum breadth of individual worms vary between 5·9 mm. broad and 0·29 mm. long and 4·5 mm. broad and 2 mm. long (!), and the same variability is displayed in the narrow anterior proglottids. Posteriorly

a few of the relatively narrow proglottids are normally longer than broad, but rarely more than twice as long. In young worms all the proglottids are broader than long. All the proglottids are fairly thick, and cannot be mounted as transparent objects displaying the internal anatomy. The strobila, when preserved in alcohol, is a creamy-yellow colour.

I possess more than two dozen scolices, two of which I have sectionised longitudinally (for observation of the hooks). The scolex is very small and chiefly consists of the four outstanding suckers and the rostellum (Fig. 1). The body of the scolex is a mere column, about 0·26 mm. broad and 0·49 mm. long (from base to extremity of rostellum). The rostellum when protruded is of the same breadth as the scolex column and is of the usual type and carries a simple single circle of minute Davaineid hooks. These hooks are very numerous and measure at a maximum about 11 microns in length. I have examined carefully seven scolices and in none could I detect a second circle of hooks. The suckers project altogether away from the body of the scolex, facing outwards, are roughly spherical in form and measure about 0·31 mm. in diameter. There is no unsegmented neck region. In the strobila the genital pores are irregularly alternate (though the unilateral arrangement holds for considerable numbers of consecutive segments) and open in each case towards the anterior end of the margin.

The arrangement of the organs in a mature proglottid is as follows. The testes are roughly about 100 in number, arranged in a vertical wall (about four layers deep in transverse sections and 24–27 in number in horizontal sections) along the posterior border of the proglottid between the two pairs of excretory canals, are individually apparently spherical in form and measure about 0·066 mm. in diameter. One curious feature concerning this wall of testes, already commented upon by Beddard (1912), is that in most mature segments the testes lie actually in the commissural excretory canal, the parenchyma containing the testes lying immediately in front of the canal protruding into it so as, in many segments, to occupy most of its lumen. However, as Beddard remarks, "though the individual testes appear at first sight to be actually bathed by the fluid of the excretory tube, a more careful examination has always shown a layer of interstitial tissue separating the actual testes from the water-vascular spaces." The testes gradually shrivel up and cease to block the commissural canal as the uterus develops. The cirrus sac, with the cirrus completely retracted, is a large oval body, the outer end reaching, in horizontal sections, the level of the ventral excretory canals, and the inner end lying half-way between the excretory canals and the margin of the proglottid. The sac measures, in this condition, about 0·28 mm. long and 0·13 mm. broad. From the outer end of the sac the cirrus (0·055 mm. broad) extends to the base of the genital cloaca, the opening of the vagina into the cloaca lying behind that of the cirrus. When the cirrus is extruded from the cloaca to the exterior (for a maximum length of nearly half a millimetre), the sac is much smaller (about 0·2 mm. long by 0·083 mm. broad), and the inner

end of the sac only reaches a little more than half-way to the ventral excretory canal of its side. The sac is quite simple in structure, a fairly thick muscular wall enclosing a cirrus tube which is straight, save at the internal end where it shows a few coils. The vas deferens, on emerging from the sac, becomes convoluted to a large extent (the coils being covered with a large number of conspicuous prostate cells) and persists in this condition until well internal to the excretory canals, when it straightens out, loses its gland cells and receives vasa efferentia from the testes. The genital cloaca is deep and dilated internally. The vagina is a straight narrow tube which emerges from the cloaca ventrally and posteriorly to the cirrus sac and runs straight to the ovary, where it joins the oviduct. Its walls are covered with small gland cells for a short distance in the vicinity of the excretory canals, and it is apparently not dilated to form a receptaculum seminis.

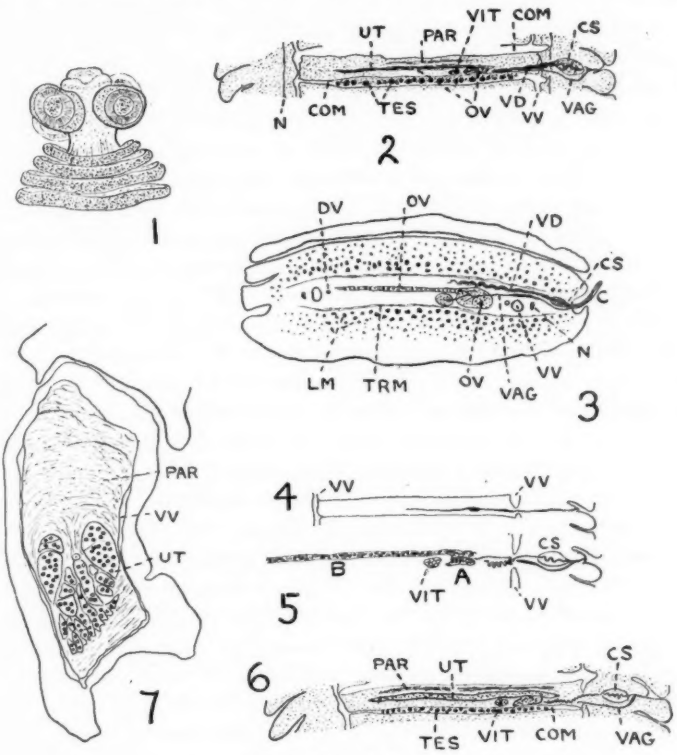
The ovary develops as a transverse strand of tissue lying in front of the line of testes, and extending across the entire breadth of the medulla between the excretory canals, but its poral end is considerably thickened, and at first I mistook this thickening alone for a poral ovary and the remainder of the streak for the uterus rudiment. But the whole of the streak contains egg-cells long before the uterus develops and the whole, therefore, must be regarded as a median ovary with a poral thickening. At a later stage the thickening gradually disappears, and the remainder of the streak develops a cavity containing eggs and becomes a transversely-elongated uterus. The uterus therefore is developed directly from the ovary. The vitelline gland is a conspicuous mass developed close to, and on the aporal side of, the poral thickening of the ovary, and disappears when the uterus develops.

The excretory system consists of the usual two pairs of canals, the large ventral pair being united transversely by a broad commissural canal at the posterior border of each proglottid, which, as already remarked, is, in mature segments, largely blocked by the intrusion of the parenchyma containing the testes. The dorsal canals are much smaller (external diameter of about 0·03 mm.) than the ventral canals (diameter of about 0·11 mm.) and thicker-walled, and lie internal to the ventral canals and slightly more dorsal. The ventral canals have "valve" flaps across their lumina at the points where they receive the commissural canals. Both vessels and the longitudinal nerves lie ventrally to the genital ducts.

The muscular system is well developed. The medulla is little more than half as thick as the cortex and is delimited from the latter by a layer of fine transverse muscle fibres. The longitudinal muscle fibres are collected into numerous bundles of very different sizes quite irregularly disposed (save that the larger bundles are more central) throughout the whole thickness of the cortex—there are no regular circles of longitudinal muscle bundles. Calcareous bodies are present throughout the parenchyma.

The transition from the mature to the fully gravid condition commences by the development of a cavity (containing the eggs or embryos) in the trans-

verse strip of ovary tissue, and almost at the same time the medullary parenchyma in front assumes a dense appearance across its entire breadth—the first appearance of the paruterine organ (Fig. 6). At this stage also the testes have shrunk in volume, and no longer appear to block wholly the commissural canal, and the poral thickening of the ovary and the vitelline gland have completely or almost disappeared. At a later stage the walls of the uterus develop ingrowths which will later sub-divide the cavity. In the final stage, previous to the shedding of the terminal proglottids from the strobila, the



Text-figures 1–7.

proglottids have elongated (Fig. 7), and the paruterine organ occupies three-quarters or more of the proglottid length and the whole of the medullary parenchyma in this region, the uterus (now largely sub-divided into large capsules, each containing a number of eggs) being restricted to the hind part of the proglottid. The spherical eggs contained in the uterus of these gravid proglottids are three-shelled (the middle shell being relatively thick) and measure in external diameter about 0·059 mm., and the hooked embryos measure about 0·044 mm. across. The hooks are about 0·022 mm. long.

Systematic position.

In many respects the present species, which I shall temporarily label with the specific name *macqueeni*, closely resembles the *Otiditaenia eupodotidis* of Beddard (1912). It resembles this species as regards the shape of the scolex, in the general arrangement of the proglottid organs and in the characters of many individual organs. The testes, muscle bundles and excretory canals are apparently similar in number and position; in both the genital pores are irregularly alternate, and in both the uterus becomes broken up into capsules each containing a number of eggs. On the other hand, there are some marked differences which indicate at least non-identity of species, such as the alternation of the hooks in the scolex ring in *eupodotidis*—a feature not present in the single ring in *macqueeni*—the presence of a receptaculum on the vagina (not present in *macqueeni*), the restriction of the ovary rudiment to the poral side of the median line (in *macqueeni* the ovary rudiment extends across the segment though there is a poral thickening, and the ovary becomes directly transformed into the uterus), the development of the whole of the medulla in front of the uterus into a paruterine organ (in *macqueeni* the formation of the paruterine organ only involves the central part of the medulla, though ultimately the whole becomes converted), the passage of the genital ducts between the dorsal and ventral excretory canals (in *macqueeni* the ducts pass dorsal to both vessels), the broader-than-long terminal proglottids and other minor differences. Now Skrjabin (1914) has concluded that Beddard's *Otiditaenia eupodotidis* from *Eupodotis kori* is identical with *Schistometra conoideis* (Bloch, 1782) (syn. *S. togata* Cholodkovsky, 1912) from *Otis tarda* and *O. arabs*, despite the fact that Beddard could not discover any tentacular appendages on the suckers of his species (*vide* Beddard, 1914; I have been unable to detect the tentacles in three scolices of *macqueeni*), that the ovary of *O. eupodotidis* is apparently not strongly poral, that the paruterine organ in this same species apparently develops initially from the whole of the medullary parenchyma and not from only a part of it, that the uterine parenchymatous capsules each contains many eggs and not one only, that Beddard's species is, judging from the published figures, only a quarter of the size of *S. conoideis* (not, it is true, an important feature by itself), and that Beddard only estimates the hooks to number about 150, whereas 400–500 is attributed to *S. conoideis*. Considering the identity of host, however, I think we must allow that Skrjabin is correct, and we may conclude (what I cannot find stated) that the genital ducts in *S. conoideis* pass between the dorsal and ventral excretory canals, as in *O. eupodotidis*. I assume provisionally therefore that the present species *macqueeni* is distinct from *Schistometra conoideis*, though closely allied. On the other hand, the present species also presents many resemblances to the tapeworm—*Ascometra vestita*—described by Cholodkovsky from the same species of Bustard, viz. *Otis* (*Houbara*) *macqueeni* from Russia. If, however, we accept Cholodkovsky's very incomplete description (1912), *Ascometra*

vestita must be regarded as generically distinct from the present species, if only because in the former the testes are stated to surround the female genitalia, and the vitelline gland is median (being situated between the two "alae" of the median ovary). In addition the dorsal excretory canal is said to be absent, the pores are unilateral and the scolex is unarmed, with a rudimentary rostellum, but these additional distinctions are hardly of generic value in view of the facts that the dorsal vessels are often absent only in the hind region of the strobila, that all intermediate conditions are to be found between the irregularly alternate and the unilateral in this group of worms, and that the Davaineid scolex can shed its hooks with great facility. Since Cholodkovsky follows his description of *Ascometra vestita* with a description of his other new species *Schistometra togata* (from *Otis tarda* and *O. arabs*), in which the testes are described as being posterior, the ovary complex as being poral and the pores unilateral, it is difficult to conclude that Cholodkovsky was mistaken in his description of *Ascometra* and we must accept the fact that *Ascometra vestita* and *macqueeni* are distinct worms, though similar in external appearance and from the same host.

The genera of the Idiogeninae.

Four genera are usually recognised as belonging to this sub-family—*Idiogenes* Krabbe, 1868; *Chapmania* Monticelli, 1893; *Sphyroncotaenia* Ransom, 1911; and *Schistometra* Cholodkovsky, 1912, and previous to attempting to assign the present species (which obviously belongs to the Idiogeninae) to one of them, it is necessary to understand clearly what are the essential characters of these genera. All species included in these genera possess the Davaineid type of scolex, but differ as regards (in addition to the more important characters given below) size and muscular development, alternate or unilateral arrangement of genital pores (the two conditions merge into each other), the presence or absence of hooks on the suckers, the dorsal excretory canal and sucker "tentacles" and the early or late passage of the eggs into the paruterine organ, and I do not consider that these features can be employed to define the genera. The diagnostic features (in combination) of *Idiogenes* appear to be the small number of testes (less than 20) and their position at the hind end of the proglottid behind the female organs, the more or less median position of the ovary complex, the passage of the genital ducts dorsal to the ventral vessel and ventral to the dorsal vessel when the latter is present, and the large cirrus sac (relative to the size of the proglottid and reaching to the middle of the proglottid or beyond). The diagnostic characters of *Chapmania* are the large number of testes and their situation either lateral to or surrounding the ovary complex (in no known species are the testes arranged solely in a sheet along the posterior border of the proglottid), the more or less median position of the ovary complex, the passage of the ducts between the ventral and dorsal excretory canals, and the small cirrus sac (relative to the proglottid breadth, and only reaching or just passing the poral excretory

canals). The diagnostic characters of *Schistometra* are the large number of testes which are arranged in a vertical sheet lying along the posterior border of the proglottid, the poral position of the ovary complex, the passage of the genital ducts between the dorsal and ventral excretory canals[1], and the small cirrus sac (relative to the proglottid breadth). The diagnostic characters of *Sphyroncotaenia* are the large number of testes distributed (in surface view) over the greater part of the proglottid, *i.e.* the testes surround the ovary complex, the ovary complex is approximately median in position (somewhat poral), the passing of the genital ducts ventral to both the ventral excretory canal and the nerve, and the relatively small cirrus sac (reaching nearly to the excretory canal).

It is evident from the preceding that the characters of the present species *macqueeni* do not accord with any one of the definitions given. As regards the number and disposition of the testes, *macqueeni* obviously belongs to *Schistometra*, but it agrees with *Idiogenes*, *Chapmania* and *Sphyroncotaenia* as regards the more or less median position of the ovary complex, and with the two latter genera and *Schistometra* as regards the size of the cirrus sac, and differs from all of them in the fact that the genital ducts pass dorsally to both excretory canals and the nerves. The only thing to do, under these circumstances, is to place the present species in a distinct genus, and I propose the name *Paraschistometra*.

Diagnosis of the new genus and species.

The definition of this new genus is as follows: Idiogeninae. Scolex of the Davaineid type. Testes numerous, arranged as in *Schistometra*. Ovary complex somewhat poral. The genital ducts are dorsal to both pairs of excretory canals and the longitudinal nerves. Cirrus sac small. Parasitic in birds.

The diagnosis of the type species, *P. macqueeni*, is as follows:

P. macqueeni. Length of strobila about 110 mm. and maximum breadth about 6 mm. Proglottids much shorter than broad except posteriorly, where some proglottids are longer than broad. Scolex very small (cir. 0·26 mm. broad and about twice as long), with armed rostellum bearing a single circle of hooks, more than 200 in number and each about 11 microns long. Suckers unarmed and very projecting; about 0·31 mm. in diameter. No unsegmented neck. Testes about 100 in number (arranged in a vertical sheet along the posterior border of the proglottid and projecting into the commissural excretory canal). Each testis measures about 0·06 mm. in diameter. Genital pores irregularly alternate. Cirrus sac measures about 0·28 × 0·13 mm. Vas deferens convoluted and covered with prostate cells. Vagina bears gland cells but is devoid of a receptaculum. Ovary stretches across the proglottid breadth in front of the testes but has a poral thickening, internal to which lies the vitelline gland. The uterus develops from the median portion of ovary by the formation of a cavity. The usual two pairs of excretory canals are present,

[1] This statement is solely based on Beddard's description of the course of the genital ducts in his *Otiditaenia eupodotidis*.

the two ventral canals being connected by a commissural canal at the hind end of each segment. The vas deferens and vagina pass dorsally to both pairs of canals and to the longitudinal nerves. Well-developed muscular system, the longitudinal bundles being irregularly scattered in a broad zone throughout the cortex. The paruterine organ occupies the anterior portion of the segment, and in elongated proglottids occupies the anterior three-quarters. The uterus become sub-divided up into capsules, each containing a variable number of eggs, and the passage of these into the paruterine organ is very late and probably only occurs in shed proglottids. The egg measures about 0·059 mm. in external diameter and the embryo about 0·044 mm. The embryo hooks measure about 0·022 mm. long. Parasitic in *Houbara macqueeni*.

Comment on the systematic position of Ascometra vestita.

If it be allowed that *Ascometra vestita* is closely allied to such forms as *Chapmania tapika* and *C. unilateralis*, then it is evident that the absence of hooks on the scolex of the former can, at most, only justify us in retaining the former in a separate genus (so following the precedent of *Taeniarhynchus* and *Taenia*). At present, however, the genus *Ascometra* is, solely on account of its unarmed rudimentary rostellum, placed in a separate family, the Hymenolepididae, sub-family Paruterininae, and so long as the absence of rostellar hooks is regarded as a more important character than the positive configuration of the proglottid organs this is inevitable. According to present usage, a Davaineid which has lost its hooks appears to be unimaginable, though this is allowed to be a possibility in the Hymenolepididae.

II. *HEMIPARONIA MEROTOMOCHETA* SP. NOV. FROM LEADBEATER'S COCKATOO.

(Text-figures 8–14.)

In the same collection of Helminths deposited in the Wellcome Bureau, I found about four more or less fragmented specimens of a tapeworm from the intestine of the common Australian Cockatoo, *Cacatua leadbeateri*, and these appear to represent a very distinct new species. This species is chiefly remarkable for its disjointed form of excretory system and the part this plays in the shedding of gravid segments.

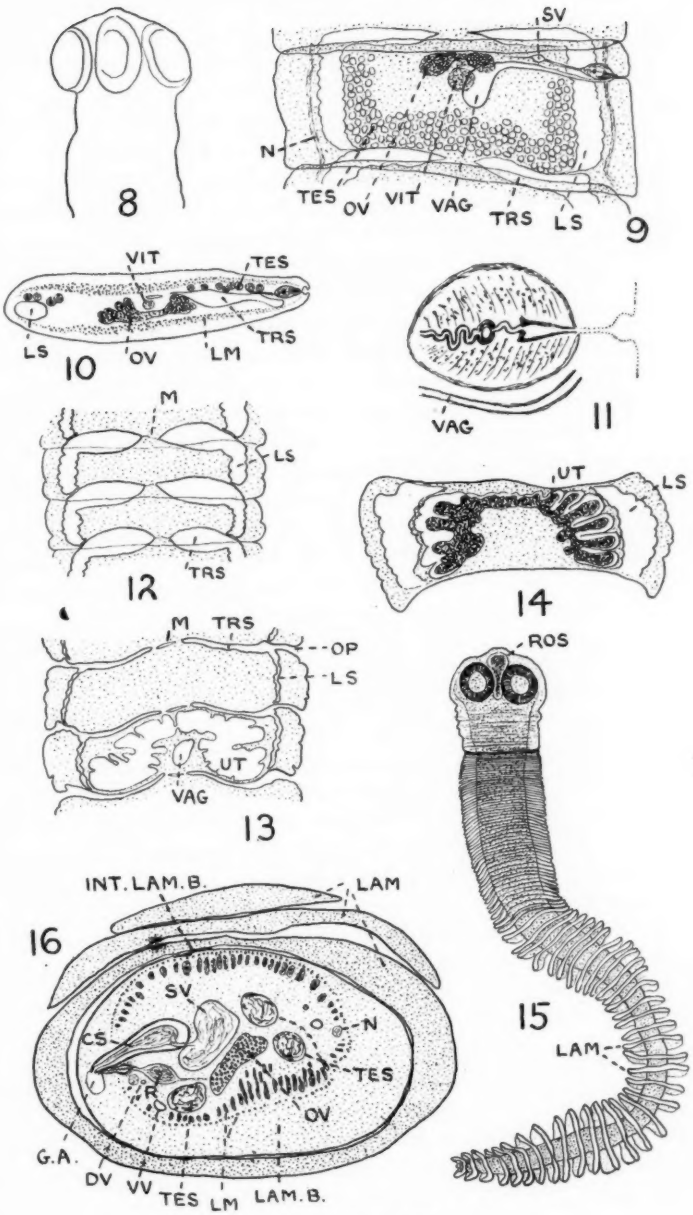
Anatomy.

The total length of the entire worm is unknown, owing to the facility with which the end segments separate from the strobila, but the minimum is 60 mm. The anterior fifth or sixth of the strobila is extremely slender (0·166–1·3 mm.), but behind this the segments rapidly broaden, and in the course of another 20 mm. or so have attained their maximum breadth of about 4 mm. The proglottids are extremely numerous and are all broader than long. The scolex (three examples) is small (about 0·026 mm. broad and 0·18 mm. long), with large laterally-placed suckers (cir. 0·128 mm. in diameter), and entirely devoid of a rostellum and of hooks (Fig. 8). The narrow strobila situated immediately behind the scolex is unsegmented for a length of about 0·8 mm.

Mature proglottids are to be observed at about 20 mm. from the scolex and they measure about 1 mm. broad and 0·3 mm. long. Gravid proglottids with large lobulated uteri full of eggs measure 2·5–4·0 mm. in breadth and about 0·5–0·7 mm. in length. The structure of a mature proglottid is as follows. The testes are numerous (about 150 roughly) and are disposed dorsally and laterally and posteriorly (in a broad transverse band) to the ovary complex, thus forming three sides of a rectangle in surface view. On the average the fully-developed testes have a diameter of about 80 microns. The genital apertures are unilateral (on the right side). The genital atrium is represented by a narrow cleft with a small dilatation at its bottom, into which open the cirrus sac (anterior) and the vagina (posterior). The cirrus sac (Fig. 11) is in mature segments a small compact ovoid body (about 212 × 131 microns in transverse sections), but in gravid proglottids it becomes longer and more narrow (256 × 73 microns). It is thick-walled, with external longitudinal muscles. The cirrus is unarmed, with a narrow lumen, and occupies nearly the outer half of the sac length. The inner end of the broad cirrus is continuous with a straight narrow canal which opens into a small internal vesicula seminalis, and this is continuous with a convoluted portion of duct which emerges from the sac as the external vas deferens. The vas deferens is narrow, runs on the dorsal side of the excretory canal and medulla, and bears an ovoid external vesicula seminalis. The vagina runs posteriorly and ventrally to the vas deferens, and ventrally to the sac. Soon after leaving the vicinity of the sac, it dilates and continues to dilate until it ends in a bulbous swelling just dorsal to the shell gland in the median line. The vagina wall is devoid of gland cells.

The ovary is situated anteriorly, medianly and ventrally, and consists of two prominent lobulated alae united by a central tubular "isthmus." Immediately posterior to the isthmus is the large median ventral vitelline gland, which is also lobulated. From the isthmus of the ovary arises the oviduct which runs dorsally and unites with the descending narrow duct from the dilated end of the vagina to form the ootype, which also receives the duct from the vitelline gland. The wall of the ootype is covered with numerous shell gland cells, the shell gland thus lying dorsal to the isthmus and ventral to the inner end of the vagina.

The excretory system is, as I have already stated, remarkable and perhaps unique in two respects, the first being that the canal system of each half of each proglottid appears to be quite separate from, though adjacent or actually apposed to, the canal system of the other half of the same proglottid and of other proglottids (the longitudinal canals thus being discontinuous segmentally), and the second being that the transversely-disposed portions of the canals take a considerable share in the process of casting-off of gravid proglottids from the hind end of the strobila. In transverse and horizontal sections of young and mature proglottids it can be seen that only a single ventral pair of canals is present, the canal of each side bending in at right angles at the anterior border of the proglottid to run transversely towards the



Text-figures 8–16.

middle line, but these transverse canals do not unite across the middle line to form a normal commissural canal, though the internal extremities appear to be joined by a thin sheet of tissue. It can also be seen, *in toto* preparations as well as in sections, that the longitudinal portions of canal of each proglottid end blindly posteriorly, though their walls are in close contact with the walls of the canals of the succeeding proglottid. At least I have not been able to detect any communications, and if they exist they must be very small. Personally, and after much searching, I believe that no such communications exist, and in view of the fact that in the older segments the transverse canals communicate with the exterior (when, if the excretory canals were continuous, the whole of the system would become empty) I think this is the correct view. I know of no other worm in which such discontinuity of the excretory system has been described. The arrangement of the canals is shown in Figs. 9–12. In gravid segments several important changes have taken place (Fig. 13). One is the establishment of openings from the outer ends of the transverse portions of the canals to the exterior; another is the formation of a split in the narrow strip of parenchyma (Fig. 13, *M*) separating the internal ends of the two transverse canals of the segment; and another is the narrowing of the canals—an obvious consequence of the communication with the exterior. The first two changes ultimately lead, by mere increase, to the complete separation of the proglottid from the proglottid in front, and in this remarkable way the segment is finally cast off from the strobila. I am not aware of the excretory system being used for such a purpose in any other Cestode. I may add that subsequent to the separation of the proglottid from the strobila the longitudinal portions of canals apparently lose their openings to the exterior and again become filled (Fig. 14).

The longitudinal nerves lie immediately external to the canals and are broad but not very distinct in transverse sections.

The medulla is only separated from the cortex by a fairly broad irregular layer of large and small bundles of longitudinal muscle fibres, transverse muscles being absent. Calcareous corpuscles are very numerous in the outer region of the cortex.

The uterus presumably arises as outpushings, one on each side, from the ootype, and its final form is shown in Fig. 14. From this it will be seen that it is of large size, the median part (*i.e.* near its seat of origin) being anterior and the lateral parts bending backwards and occupying the bulk of the medulla. In form it is extremely lobulated, and in places the peripheral lobules extend external to the excretory canals. In my preparations it is filled with numerous small (14–18 microns in diameter) spherical thin-shelled eggs, containing multinucleated embryos devoid of hooks. In completely gravid proglottids all the genital organs have disappeared with the exception of the uterus, the cirrus sac and the vagina, the last being very large and filled with spermatozoa, its inner end lying behind the median part of the uterus, but its outer part extending in front of the right half of the uterus. Further, the external clefts

between the segments have deepened so as almost or completely to meet across the middle line, and the ripe proglottids freely separate from the main strobila. In virtue of the peculiar condition of the excretory system in this worm, I propose the specific name *merotomocheta*.

Systematic position.

Though I am not aware of any one character or combination of characters possessed by the present worm which would justify us in referring it to the Anoplocephalidae rather than to the Hymenolepididae, yet this form evidently belongs to the genus *Hemiparonia*, recently created by Baer (1925) to accommodate the tapeworm (originally named *Schizotaenia cacatuae*) found by Maplestone (1922) in a common Australian Cockatoo (*Cacatua galerita*) closely allied to *Cacatua leadbeateri*. Like the hosts, the two tapeworms are thus species of the same genus, and *H. cacatuae* (Maplestone) only differs from *H. merotomocheta* in such relatively unimportant characters as the greater length (relative to breadth) of many of the mature proglottids, the presence of a layer of transverse muscle fibres underlying the layer of longitudinal bundles, and the presence of dorsal excretory vessels. On the other hand, both species agree in possessing all the more important features, such as the unarmed scolex, unilateral pores, genital ducts passing dorsally to the excretory canals, the single layer of longitudinal muscles, the number and position of the testes, the median ovary, vitelline gland and receptaculum seminis and the form of the uterus, and, indeed, further examination may even show that *H. cacatuae* agrees with *H. merotomocheta* in possessing a similar disjointed form of excretory system, if we may judge by Maplestone's remarks (1922, p. 307) concerning "variations" of the vessels and Baer's Fig. 13 (1925).

The diagnosis of *Hemiparonia merotomocheta* is as follows: strobila 60 mm. and more in length, with the anterior fifth or sixth extremely slender. Maximum breadth (posteriorly) about 4 mm. Proglottids very numerous, all broader than long, and the hind segments readily separate from the strobila. Scolex small (about 0·026 mm. broad and 0·18 mm. long) with large lateral suckers (0·128 mm. diameter). Short neck present. Testes about 150 in number, dorsally placed and posterior and lateral to the ovary complex, forming three sides of a rectangle. Cirrus sac small and compact, with unarmed cirrus and small internal vesicula seminalis. Vagina dilated internally and devoid of gland cells. Ovary and vitelline gland both large and lobulated. Only two ventral excretory canals present, each with a transverse extension anteriorly. The canal system of each half of each proglottid entirely separate from that of the other half and from those of other proglottids. Longitudinal muscle bundles variable in size, forming a thick layer. Transverse muscles absent. Calcareous corpuscles numerous in outer layer of cortex. Uterus large and lobulated and curved in form, the middle part being anterior and the lateral parts extending posteriorly. Eggs numerous, thin-shelled and spherical (about 14·18 microns in diameter). Completely gravid proglottids easily separate from strobila. Host and locality: *Cacatua leadbeateri* (intestine), Australia.

III. *Hymenolepis lamellata* sp. nov. from the Australian Sheld-drake.

(Text-figures 15, 16.)

I possess three specimens and the greater part of a fourth of this Cestode, but only one is entire (the strobila of two of the others being broken posteriorly). The most characteristic feature of this new species of *Hymenolepis* —*H. lamellata*—is its striking Polychaete-like appearance, the short narrow proglottids each bearing a prominent regular lamella-like expansion of its periphery on all sides. This material was derived, I believe, from a bird which died in the London Zoological Gardens, and was described on the label accompanying the worms as an "Australian Sheld-Drake." I am ignorant as to whether this description signifies *Tadorna radjah* or *Casarca tadornoides*, but probably the former.

Anatomy.

The worms (fully mature) had a maximum length of about 11 mm. and a maximum breadth (fairly uniform except posteriorly) of about 1 mm. (including the lamellae). The central or axial strobila (*i.e.* without the lamellae) has a maximum breadth (anteriorly) of about 0·7 mm. Though the lamellae give the strobila a cylindrical appearance, yet the axial portion of strobila which bears them is slightly flattened dorso-ventrally (about 0·54 mm. dorso-ventrally and 0·69 mm. laterally) and more so posteriorly. The lamellae measure in breadth about half the depth of the axial strobila. The scolex, in the region of the suckers, is broader than the strobila (1·27 mm. in one case), though behind the suckers it is of the same breadth (Fig. 15). It is longer than broad (1·37 mm. in the same case) and carries a distinct eversible rostellum which, however, is devoid of hooks (a statement based on one intact scolex and two in sections). The suckers are globular and measure about 0·4 mm. in diameter. The line of division between the base of the scolex and the strobila is sharp, and there is no unsegmented neck region. The portion of strobila immediately succeeding the scolex is (without the lamellae) narrower than the scolex and the proglottids are exceedingly short in proportion to their breadth, being only as thick as the lamellae. At about a quarter of the length of the strobila the proglottids become relatively long (0·13 mm., corresponding to a breadth, without lamellae, of 0·28 mm.) and they more or less maintain these dimensions posteriorly, though, owing to the extreme posterior lamellae becoming shorter, they appear to elongate considerably in the last 2 mm. of the worm's length.

The internal anatomy of mature proglottids is quite typical of the genus *Hymenolepis*. The genital pores are unilateral (and on the right side). The testes are of course three in number and large (cir. 0·128 mm. in diameter in transverse sections). Two testes are situated, one above the other, aporally, and one porally on the ventral side, ventral to the cirrus sac and external

vesicula seminalis. The small genital atrium opens on the anterior side of the lamella at a point about one-third of its depth from its base, and into it open the cirrus sac and vagina (ventral to the sac). The sac is flask-shaped (bulbous at its inner end) and extends a little internally to the two excretory canals. The outer half of the cirrus bears numerous closely-set spinelets, and the vas deferens bears, in addition to the large conspicuous external vesicula seminalis already mentioned, a small internal vesicula inside the sac. Both the sac and the vagina pass dorsally to the two excretory canals and the poral nerve. The vagina bears a large receptaculum seminis situated above the poral testis and below the vesicula seminalis. The lobed ovary and vitelline gland are situated medianly or very slightly porally and ventrally to the vesicula seminalis.

The main excretory system consists of the usual two pairs of canals; transverse commissural canals appear to be absent.

The cortical parenchyma is wholly contained in the lamellae, the surface of the strobila between the lamellae overlying the medulla only; in other words, the cortex is absent between the lamellae, and the longitudinal muscle bundles are only separated from the cuticle by the very thin sub-cuticula. The longitudinal muscles consist of an external row of small bundles (lying immediately underneath the sub-cuticula in the interlamellar regions) and an inner row of larger elongated bundles radially disposed. The longitudinal nerves are large and are situated between the two excretory canals of each side and the longitudinal muscles. In the anterior immature region of the strobila the longitudinal muscle bundles are all elongated and radially disposed and are much larger than those situated more posteriorly; still more anteriorly, just behind the scolex, the longitudinal muscle fibres are not concentrated into bundles.

In gravid segments (which I have only been able to study in vertical longitudinal sections) the sac-shaped uteri are situated towards the dorsal surface, are well developed (I have no information as to their mode of origin) and contain numerous spherical thin-shelled eggs measuring about 22 microns in diameter. The vesiculae seminales are very large in gravid segments and alternate dorso-ventrally in longitudinal sections for economy of space. The cirrus sacs, vaginae and receptacula also persist.

IV. CORRECTION.

In a paper "On some new Avian Cestodes from the Sudan," recently published in *Parasitology* (**20**, 3, 1928, p. 305), I have described a new species of Cestode from *Numida ptilorhyncha*, which I left unnamed. Prof. Meggitt has kindly suggested to me that I may have overlooked the "prong" of the hook head which, if present, would be situated opposite to that figured, the hook thus, with this addition, being of the ordinary Davaineid type, though large in size. I have ascertained that this is actually the case, and the worm described is therefore a new species either of *Raillietina* or of *Ophryocotyloides*,

and is chiefly characterised by hook size and the peculiar formation of the suckers.

I wish to express my indebtedness to Miss I. Bellis for assistance in translation and to Dr C. Hoare for similar aid in connection with Cholodkovsky's original memoir in Russian.

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EXPLANATION OF TEXT-FIGURES 1–16.

(All figures drawn under the camera lucida.)

Figs. 1–7. *Paraschistometra macqueeni.*

Fig. 1. (×27·5): the scolex.

Fig. 2. (×17·5): composite drawing of a horizontal section of a mature proglottid.

Fig. 3. (×12): transverse section of mature proglottid.

Fig. 4. (×17·5): early stage of development of the ovary and vagina (as seen in horizontal sections).

Fig. 5. (×17·5): later stage of development of the same. *A* and *B* represent respectively the thick poral portion of the ovary and the thin extended central portion, *B* alone becoming transformed directly into the uterus.

Fig. 6. (×17·5): composite drawing of horizontal section of a proglottid in which the central portion of the ovary has become an elongated sac-shaped uterus containing eggs, distinct from the poral ovary thickening.

Fig. 7. (×12): completely gravid proglottid in horizontal section.

Figs. 8–14. *Hemiparonia merotomocheta.*

Fig. 8. (×87·5): the scolex in outline.

Fig. 9. (×56): a mature proglottid (*in toto* preparation).

Fig. 10. (×17·5): transverse section of young mature proglottid.

Fig. 11. (×180): the cirrus sac in a transverse section.

Fig. 12. (×27·5): the disjointed excretory canals as seen in a young mature proglottid mounted *in toto*.

Fig. 13. (×12): outlines of gravid proglottids in which the transverse excretory canals have opened to the exterior at the margins and will shortly coalesce with the median split (*M*) preparatory to the separation-off of the proglottid from the strobila. The longitudinal canals now communicate with the exterior and are in consequence very reduced in size.

Fig. 14. (×17·5): gravid proglottid which has separated from the strobila (the longitudinal canals have lost their openings to the exterior and are filled again).

Figs. 15–16. *Hymenolepis lamellata.*

Fig. 15. (×12): the worm as a whole, showing the conspicuous lamellae.

Fig. 16. (×56): composite drawing of a transverse section. It will be seen that in the interlamellar region (*INT. LAM. B.*) the medulla extends to the extreme margin of the proglottid and that therefore the whole of the cortex is contained in the lamellae (*LAM. B.*).

REFERENCE LETTERS.

A, poral thickening of ovary; *B*, median portion of ovary; *C*, cirrus; *COM*, commissural excretory canal; *CS*, cirrus sac; *DV*, dorsal excretory canal; *G.A.*, genital atrium; *INT. LAM. B.*, interlamellar edge of proglottid; *LAM*, lamellae; *LAM. B.*, lamellar border of proglottid; *LM*, longitudinal muscles; *LS*, longitudinal portion of excretory canal; *M*, sheet of tissue (later developing a cavity) uniting the inner ends of the transverse portions of the excretory canals; *N*, longitudinal nerve; *OP*, opening to exterior of excretory canal; *OV*, ovary; *PAR*, paruterine organ; *R*, receptaculum seminis; *ROS*, rostellum; *SV*, vesicula seminalis; *TES*, testis; *TRM*, transverse muscles; *TRS*, transverse portion of excretory canal; *UT*, uterus; *VAG*, vagina; *VD*, vas deferens; *VIT*, vitelline gland; *VV*, ventral excretory canal.

(*MS. received for publication* 1. x. 1929.—Ed.)

THE ACTION OF HYDROCHLORIC ACID UPON CULTURES OF *TRICHOMONAS*.

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Introduction.

The question of the mode of transmission of the *Trichomonas* of man from one host to another is of particular interest because no authentic encysted stage is known.

Many workers have attempted to infect "clean" animals by the mouth with active trichomonads from a variety of hosts, either from cultures or directly from faeces. The results are conflicting. Thus Escomel (1913) fed faeces containing *Trichomonas intestinalis* (= *T. hominis*) from a human case of dysentery to a rabbit, a guinea-pig, a cat, and a dog, and obtained a positive result in the dog. He does not, however, state that he examined his animals, to find whether they had any similar natural infections, before performing his experiments. Boyd (1919) fed a culture of *Trichomonas intestinalis* (= *T. hominis*), mixed with milk, to a white rat and infected it. He had previously examined this rat and one used as a control and found them to be free from this flagellate. He was, himself, uncertain whether the infection was due to the free flagellates or their "cysts" which he says were present in the culture. The cysts figured by him (Plate XI, figs. 7–17) appear to be rounded-up and degenerating trichomonads. Pringault (1920) fed *Trichomonas intestinalis* (= *T. hominis*) to a cat, a guinea-pig, a rabbit, and a white rat, without establishing an infection in any one of them.

Wenyon (1907) studied the direct action of gastric juice (from a freshly killed mouse) upon *Trichomonas intestinalis* (= *T. muris*) from the faeces of a mouse. He found that the trichomonads, which had been rounded-up and quiescent (the moist faeces containing them had been kept for several days), revived, and remained alive for 4 or 5 hours. He concluded that it was quite possible for infection to occur by the ingestion of *Trichomonas* in an unencysted condition.

Hegner (1924) fed *Trichomonas muris* to rats and examined the contents of the stomach and duodenum at varying intervals afterwards. He found active trichomonads in the stomach of three rats at least 1 hour after feeding. That the passage through the stomach is sometimes rapid was shown by his finding living trichomonads in the duodenum half-an-hour after feeding them to the rat. As a result of a number of experiments he found that the flagellates could pass through the stomach and 780 mm. of small intestine without apparent injury. Later (1926) he found that *Trichomonas caviae* and *T. flagelliphora* from one guinea-pig could pass, apparently unharmed, through the stomach to the caecum in another guinea-pig. He noticed that movement was partially inhibited in the stomach and the anterior part of the small intestine, but it became more normal as the caecum was approached.

Brumpt (1925) experimentally infected cats and dogs, which he had examined previously and found to be negative for *Trichomonas*, with *Trichomonas felis*, by the mouth. Kessel (1928) succeeded in infecting a number of "clean" kittens *per os* and *per anum* with *Trichomonas* from different hosts. His diagnosis of "clean" was based upon negative microscopic examinations of faeces obtained by an enema, and of cultures of the faeces, made 3 days in succession. His results of the infection by mouth are of interest here. Four kittens out of six were infected with *Trichomonas hominis*; two with cultures of *Trichomonas* from naturally infected kittens; one out of two kittens was infected with *T. parva* from the caecum of white rats; and one of two kittens was infected with a *Trichomonas* from *Macacus rhesus*. Wenrich and Yanoff (1927) fed caecal contents containing *Tritrichomonas muris*, *T. minuta*, *T. parva*, and *Pentatrichomonas* sp., all parasites of the rat and all in an active state, to rats. They also fed a culture of *Pentatrichomonas* of man to rats. They found that these flagellates could pass through the stomach into the small intestine.

Of interest in this question of the establishing of an infection with unencysted *Trichomonas* by passage through the stomach are the fractional gastric tests made by Tsuchiya (1925) upon a number of patients infected with *T. intestinalis* (= *T. hominis*). He found that hypochlorhydria was frequently associated with the presence of *Trichomonas*. He was not certain that this deficiency is a result of the infection, but considered that it might arise from metabolic and nutritional disturbances due to the other pathological conditions of the patients. Lèvy (1922) also noted a marked hypochlorhydria in cases infected with *Trichomonas* and also an absence of bile in the faeces.

Mr Dobell has pointed out to me that the percentage of achlorhydric persons, in England, is approximately equal to the percentage infected with *Trichomonas*. As *T. hominis* has no known encysted stage, a lack of acid secretion in the stomach of the future host would facilitate the passage of the flagellate through the stomach. Therefore, instead of regarding hypochlorhydria as a result of an infection with *Trichomonas*, it is possible that this deficiency of gastric juice, due to some clinical condition apart from the

presence of the flagellate, may have assisted, or made possible, the infection with *T. hominis*.

The following experiments were made with the object of finding the average and the maximal time of survival of the trichomonads of man and of macaques when acted upon directly by hydrochloric acid in concentrations similar to that of normal human gastric juice. I wish to offer my thanks to Mr Clifford Dobell for proposing the problem, for providing the strains of *Trichomonas*, and for the help he has given me; also to Dr P. P. Laidlaw for the advice he gave me upon the determination of the *p*H of the liquids.

Material and methods.

Three strains of *Trichomonas*, all isolated by Mr Dobell, were used for these experiments: a strain (*F.T.*) of *Trichomonas hominis* isolated from human faeces on August 25, 1925, and cultivated upon inspissated horse-serum slopes covered with egg-white diluted (1 : 8) with Ringer's fluid (Dobell and Laidlaw, 1926); a *Trichomonas* (strain *T.*) isolated *post-mortem* from a *Macacus rhesus* on May 5, 1925, and cultivated in the same medium; and a *Trichomonas* (strain *N.T.*) isolated at the *post-mortem* examination of a *Macacus nemestrinus* on August 3, 1927, and cultivated upon coagulated whole-egg slopes covered with inactivated horse-serum in Ringer's fluid (1 : 8)[1]. Each of these three strains gave excellent cultures which needed sub-cultivating only every 5 or 7 days. For the purpose of these experiments 36-hour or 48-hour cultures were used. At this age the tubes contain a thick growth of trichomonads whilst the number of bacteria present is still small. Older cultures become thick and slimy—which might prevent the uniform action of the acid.

The experiments were made both at room-temperature (15° C.–22° C.) and at body-temperature (37° C.). Cultures of well-established strains of *Trichomonas* will survive for many days at room-temperature. The ability of *T. hominis* to live at room-temperature has been mentioned by a number of workers. Thus, Dobell and O'Connor (1921, p. 70) kept this species alive in liquid stools for periods up to a month. Faust (1921) found that daily examinations of an infected stool were positive until the eleventh day. Hegner and Becker (1922) found that cultures made from a stool 72 hours old were positive for *Trichomonas*, but smears made from the stool were negative after 37½ hours. Kofoid and Swezy (1923) found that *Pentatrichomonas* disappeared from an infected stool in 3 to 5 days; but they found living individuals in a very liquid stool after 24 days. Hegner (1928) obtained positive cultures from faeces kept at room-temperature for 8 days, but he found that his cultures in serum-saline-citrate became negative after 2 days at room-temperature.

[1] No attempt has been made to give specific names to the *N.T.* and *T.* strains of *Trichomonas*, as their actual systematic relationship is not yet definitely determined. They differed in slight morphological details. A note upon the presence of the *Trichomonas* in *Macacus rhesus* has been published by Mr Dobell (1925); and a later reference (1928) has been made to the *Trichomonas* found in *M. nemestrinus*.

For the experiments carried out at 37° C. the Institute "hot room" (kept at 37° C.) was used, and all the apparatus required was arranged there overnight, in order that no cooling should occur at any stage of the experiment.

The following general method was adopted. A culture of *Trichomonas* was well stirred up and a definite quantity of the fluid removed to a sterile tube in a sterile graduated pipette. The volume of acid (*N*/10 HCl or *N*/30 HCl) required to bring the concentration of the whole to *N*/20 HCl and *N*/40 HCl was then added. The mixture was stirred, care being taken to prevent it swilling up the sides of the tube. The *p*H of the mixture was determined, and when the requisite time had elapsed the liquid was neutralized with a sterile solution of sodium bicarbonate—using sterile neutral red as an indicator. The flagellates and suspended particles were allowed to sink, and a little of the sediment was examined microscopically for trichomonads, and some of it was inoculated into a tube of culture-medium containing a growth of bacteria which had previously been proved favourable to the growth of the trichomonads. During the whole process great care was taken to avoid any contamination.

EXPERIMENTAL.

In the first series of experiments 1 or 2 c.c. of *N*/10 HCl was added to an equal volume of culture, giving a final concentration of *N*/20 HCl. In the earlier and greater number of these experiments the *p*H was not determined, but later it was determined colorimetrically by the drop method, using Thymol blue and Bromphenol blue as indicators. The *p*H was taken after the addition of the acid, and—with the exception of one experiment with the *T.* strain in which the *p*H was 4·0—lay between 2·0 and 2·5. (In thirty experiments the *p*H was found to be 2·0, in twenty-two it was 2·25, and in seven 2·5.) The variation in *p*H, within this range, did not appear to bear any relation to the time of survival.

The experiments were done both at room-temperature (Table I) and at 37° C. (Table II). The positive and negative results are based upon the examination of 2-day old cultures made from the neutral sediment. With three exceptions the results of the direct microscopic examinations of the sediment were always negative. In these three, a few active trichomonads were found in a preparation made from the *N.T.* strain after 5 minutes in *N*/20 HCl at room-temperature; one motile *Trichomonas* was discovered in the *F.T.* strain treated with *N*/20 HCl for 45 minutes; and three motile trichomonads were found in a preparation of the *T.* strain which had been treated with *N*/20 HCl for 60 minutes.

The amount of visible change undergone by the trichomonads when treated with the acid varied considerably. In preparations made during the majority of the experiments the flagellates were very contracted and shrivelled. They were motionless, and the nucleus often appeared as a dark ring at the anterior end of the animal. In two experiments many of the trichomonads appeared to be less damaged by the action of the acid, and these, in both cases, gave positive results.

Experiments were made to determine whether the distilled water, used for the greater number of experiments for diluting the normal HCl, was toxic in any degree to the trichomonads. Small quantities of cultures of the three trichomonads were diluted with 5 c.c. of sterile distilled water, and sub-cultures afterwards made from time to time up to 2 hours, and examined 2 days later. Whereas the sub-cultures from the *N.T.* strain and the *F.T.* strain were always positive those from the *T.* strain gave variable results. It would appear therefore that sterile distilled water is slightly toxic to this strain of *Trichomonas*. Hegner (1928) found whilst experimenting with dilutions of faeces containing *Trichomonas hominis* in tap and distilled water, that the diluent had an adverse effect upon viability. He attributed this principally to changes in the osmotic pressure of the medium. Kofoid and Swezy (1923) found earlier that faecal material diluted with sterilized tap-water, rain-water, or creek-water, was less favourable for the survival of *Trichomonas* than undiluted faeces.

Since distilled water, as a diluent, did not give constant results in all three strains, Ringer's solution was tested in the same way. Excellent cultures were obtained from all the three strains up to 2 hours, which was the maximum time of the tests. The *N*/10 HCl in Ringer was tested with Thymol blue against the *N*/10 HCl in distilled water. The *p*H was the same.

A few experiments were therefore made with the three strains of *Trichomonas* with *N*/20 HCl in which the normal HCl was diluted to *N*/10 HCl with Ringer's solution. But in comparing these results with those in which the diluent was distilled water no difference in the time of survival, attributable to the nature of the diluent, could be discovered.

The duration of the experiments varied from a mere shaking-up of the acid mixture and immediate neutralization, to a lapse of 90 minutes between the addition of the acid and the neutralization of the mixture.

In the experiments made at room-temperature (Table I) it will be seen that, whereas positive cultures were obtained in the *N.T.* strain after 45 minutes' immersion in *N*/20 HCl, in the *F.T.* strain after 75 minutes and in the *T.* strain after 60 minutes, in the two former strains the number of negative cultures became large at 30 minutes, whilst in the *T.* strain 15 minutes' immersion

Table I.

Experiments at room-temperature with N/20 *HCl.*

Time in min.	Number of cultures		
	Strain *N.T.*	Strain *F.T.*	Strain *T.*
5	2 +	1 +	2 +, 2 −
10	—	—	1 +, 3 −
15	12 +	7 +, 4 −	3 +, 18 −
30	7 +, 17 −	6 +, 20 −	3 +, 19 −
40	3 +, 1 −	1 +	2 −
45	2 +, 9 −	3 +, 10 −	2 +, 7 −
60	3 −	1 +, 3 −	2 +, 4 −
75	2 −	1 +, 3 −	2 −
90	—	2 −	—

Table II.

Experiments at 37° C. with N/20 HCl.

Time in min.	Number of cultures		
	Strain *N.T.*	Strain *F.T.*	Strain *T.*
At once	—	—	1 +
5	1 +, 4 −	2 +, 2 −	6 −
10	3 −	3 −	2 −
15	2 −	1 +, 1 −	2 −
30	1 −	1 −	1 −
45	2 −	2 −	2 −
60	2 −	1 +, 2 −	2 −

produced a large proportion of negative results. The experiments made at 37° C. (Table II) showed that the action of *N*/20 HCl even for 5 minutes was lethal in some cases, whilst 10 minutes—with the exception of two experiments made with the *F.T.* strain—invariably killed. Concerning the positive result obtained with the *F.T.* strain after 60 minutes at this temperature and concentration of acid it is possible that—as this was a very early experiment—one trichomonad (or perhaps a few) became accidentally swilled up the side of the tube and so was not subjected to the full concentration of the acid. After this the greatest care was taken to obviate this possible error.

It is to be supposed that, in cases of natural infection, the trichomonads, assuming that they pass from host to host in the active flagellate stage, would undergo cooling and be subjected for a varying length of time to room and outdoor temperature before gaining access to their new host. In order to discover whether a period at room-temperature made the flagellates more resistant to the action of the acid, cultures were left on the bench 24 hours before the acid was added. When the experiments were to be performed at 37° C. the cultures were warmed to that temperature immediately before the acid was added. The results of these experiments were, in every case, negative, indicating that cooling does not add to the acid resistance of the flagellates, but, on the contrary, tends to lower it.

In order to discover whether cultures from trichomonads which had survived the action of the acid were more resistant than ordinary cultures, such cultures from positive experiments were treated with *N*/20 HCl, but these did not give a greater number of positives than ordinary cultures treated in the same way.

As the survival time of *Trichomonas* in *N*/20 HCl is, on the whole, so low, particularly when the experiment is carried out at body-temperature, similar experiments with *N*/40 HCl were made to discover whether the time of survival at this concentration was much greater than in *N*/20 HCl. In the first series of these experiments one part *N*/10 HCl was added to three parts of culture. In the early experiments the *p*H was not estimated, but in later ones it was found to lie between *p*H 3·75 and 4·5, only one experiment of those in which the *p*H was determined giving *p*H = 3·75, eleven experiments *p*H = 4, one *p*H = 4·25,

and four pH = 4·5. These experiments were all performed at room-temperature. The results are given in Table III.

Table III.

Experiments with N/40 *HCl* (3 *parts culture* + 1 *part* N/10 *HCl*) *at room-temperature.*

Time	Number of cultures		
	Strain *N.T.*	Strain *F.T.*	Strain *T*
30 min.	—	1 +	—
45 ,,	1 +	—	—
60 ,,	1 +	1 +	—
75 ,,	—	1 +	—
90 ,,	—	1 +	1 +
105 ,,	2 +	1 +	1 +
2 hr.	—	2 −	1 +
2¼ ,,	—	—	1 +
2½ ,,	1 +	—	1 +
3 ,,	1 −	1 +, 1 −	1 +, 1 −
3½ ,,	1 +, 1 −	—	2 +
4 ,,	—	1 +	—

It will be seen that, at this concentration, positive cultures of all three strains of *Trichomonas* were obtained after a much longer action of the acid —and that very few negative results were obtained for the times tested. Whilst examining preparations of sediment from experiments with $N/20$ HCl living trichomonads were found on only three occasions, though cultures made from the apparently negative sediment were sometimes positive. This indicates that—when a positive culture is obtained—few trichomonads, or perhaps only one, withstood the action of the acid, and that it was a matter of chance whether a living one was found during the direct microscopic examination of the sediment. Support was given to this hypothesis by an experiment made at room-temperature upon the *T.* strain of *Trichomonas* in $N/40$ HCl. To one part $N/10$ HCl three parts of culture of the flagellate were added. After 1½ hours the pH was determined and found to be 4·5. Examinations of the sediment were made from time to time before the acidic solution was neutralized. In the examination made 1¾ hours after the addition of the acid only dead trichomonads were found; but two living trichomonads were found in the sediment examined after 2½ hours. An examination made after 3½ hours was again negative. On the second day the culture made from the neutralized sediment contained a rich crop of *Trichomonas*, proving the presence of living individuals, in spite of their apparent absence in the sediment examined before neutralization.

During a similar experiment with the *N.T.* strain of *Trichomonas* examinations were made at frequent intervals over a period of 3½ hours. After the first three-quarters of an hour living flagellates became very scarce. These differed amongst themselves in size—the rate of death evidently not being correlated with the size of the individual.

In the above experiments with $N/40$ HCl the volume of culture fluid was

greatly in excess of the volume of the acid. Under such conditions part of the acid would be neutralized by the culture fluid. A series of experiments was carried out in which the theoretical concentration $N/40$ HCl was the same, but three parts of $N/30$ HCl (using either distilled water or Ringer's solution as the diluent) were added to one part of culture. The results of these experiments are given in Tables IV and V.

Table IV.

Experiments at room-temperature. Trichomonas *in* N/40 *HCl* (1 *part culture* + 3 *parts* N/30 *HCl*).

Time in min.	Number of cultures		
	Strain *N.T.*	Strain *F.T.*	Strain *T.*
15	6 +, 6 −	2 +, 6 −	17 −
30	2 +, 6 −	1 +, 7 −	1 +, 4 −
45	2 +, 1 −	1 +, 3 −	3 −
60	1 −	1 −	—
90	1 −	1 −	1 −

Table V.

Experiments at 37° *C.* Trichomonas *in* N/40 *HCl* (1 *part culture* + 3 *parts* N/30 *HCl*).

Time in min.	Number of cultures		
	Strain *N.T.*	Strain *F.T.*	Strain *T.*
5	2 +, 1 −	1 +, 2 −	3 −

The pH lay between 2·25 and 3·25. In seventy-nine experiments the pH was as follows: in two experiments $p\text{H} = 2{\cdot}25$; in sixteen $p\text{H} = 2{\cdot}5$; in fourteen $p\text{H} = 2{\cdot}75$; in thirty-three $p\text{H} = 3{\cdot}0$; in fourteen $p\text{H} = 3{\cdot}25$. A comparison of Tables III and IV shows a great difference in the time of survival of the trichomonads, those in the first series with $N/40$ HCl living much longer than those in the second. Much greater similarity exists between the results of the experiments in which the trichomonads were treated with $N/20$ HCl (1 part culture + 1 part $N/10$ HCl—Tables I and II) and the second series with $N/40$ HCl (1 part culture + 3 parts $N/30$ HCl—Tables IV and V) than those of the first series with $N/40$ HCl (3 parts culture and 1 part $N/10$ HCl—Table III). The comparison of the tables, however, reveals the rather disconcerting fact that more positives were obtained from the experiments with $N/20$ HCl than from the last series with $N/40$ HCl. It is quite probable that an explanation of this lies in the relative volumes of culture, and therefore the number of flagellates used in the two sets of experiments. In the second series of experiments with $N/40$ HCl (Tables IV and V) the number of flagellates used was approximately equal to that in the experiments with $N/20$ HCl, since equal volumes of cultures of the same age were used in both sets of experiments. But in the experiments with $N/40$ HCl (second series) the final volume (8 c.c.) of fluid through which the flagellates were dispersed was double that used in the experiments with $N/20$ HCl (4 c.c.). The sediment was not

spun down, but allowed to sink for 10 minutes and the lower layer inoculated into the culture medium. The chances therefore of picking out the few individuals surviving, either for direct microscopic examination, or for sub-cultures, were much smaller in the second series of experiments with $N/40$ HCl than in those with $N/20$ HCl. An illustration of this was found in an experiment made with the *N.T.* strain of *Trichomonas*. To 2 c.c. of culture 6 c.c. $N/30$ HCl were added, stirred, and left for 15 minutes. The *p*H was 3·0. After neutralization all the trichomonads found in the preparation of sediment were dead. Three sub-cultures were made. When these were examined 2 days later one of them showed an excellent growth of *Trichomonas* whereas the other two were negative. In two similar experiments with the *F.T.* and *T.* strains all the sub-cultures were negative.

Another indication that the explanation lies in the small number of survivors and in the relative volumes of fluid used was found in another experiment with the *N.T.* strain. To 2 c.c. of culture fluid 2 c.c. of $N/10$ HCl were added and left for 15 minutes. The *p*H was 2·25. After neutralization the sediment was examined microscopically, but no living trichomonads were found. Then 4 c.c. of sterile distilled water were added thus bringing up the volume of the fluid to that used in the second series of experiments with $N/40$ HCl. The mixture was allowed to stand for a short time and two sub-cultures made from the sediment. On the second day one was positive and one negative. It is to be presumed that a living *Trichomonas* was put into one tube and only dead ones into the other.

It seems probable therefore that the difference in time of survival shown in Tables III and IV, between the first series of experiments with $N/40$ HCl in which the volume of culture to acid was as 3 : 1 and the second series in which it was as 1 : 3, may be due, not solely to the difference in *p*H and the direct action of the acid upon the flagellates, but also to the greater number of trichomonads present in the former.

The effects of desiccation upon *Trichomonas*.

A series of experiments was performed with the three strains of *Trichomonas* to discover whether they would withstand drying, a fate which seems quite probable to occur in nature. Pieces of sterile coverslips were smeared with sediment from 2-day old cultures containing thick growths of *Trichomonas*. The pieces of coverslip were placed in a desiccator until they were dry. They were then dropped into tubes containing the favourable mixture of bacteria. The average time taken for the smears to dry completely was from 1 to $1\frac{1}{2}$ hours. The experiment was repeated nine times with the *T.* strain of *Trichomonas*, seven times with the *F.T.* strain, and seven times with the *N.T.* strain. The cultures were examined on the second day and in every case were negative for *Trichomonas*. Slips smeared and dried in a similar way but put into sterile culture tubes yielded a thick growth of bacteria but no trichomonads.

It is to be concluded, therefore, that *Trichomonas* from man, *Macacus rhesus*, and *M. nemestrinus* cannot withstand drying.

DISCUSSION.

The above experiments with $N/20$ HCl and $N/40$ HCl upon cultures of *Trichomonas* from man, *Macacus rhesus*, and *M. nemestrinus* have shown that this flagellate can seldom withstand the action of acid at these concentrations at body-temperature for longer than 5 minutes. If the experiment is performed at room-temperature, which would not, of course, occur under natural conditions, the resistance of the flagellate is much greater, positive results being frequently obtained at all times up to 30 minutes after addition of the acid. A few positive results have been obtained, at room-temperature, in experiments with $N/20$ HCl in which the pH was not determined, up to 75 minutes' exposure. In experiments at room-temperature in which the pH lies above 3·75 (Table III), the flagellates can survive for a much longer period.

This result, that the acid is more lethal to *Trichomonas* at body-temperature than at room-temperature, is in contrast with the results obtained by Mr Dobell (1927) for the action of $N/20$ HCl upon the amoebae of *Entamoeba histolytica*. He found that, whereas *E. histolytica* was usually killed at room-temperature, after only a few minutes' exposure to $N/20$ HCl, it could withstand the action of the acid for an hour at 37° C. He considers that this shorter period of survival of *E. histolytica* in acid at room-temperature is due to the cooling itself being harmful to the amoebae and thus accelerating the lethal action of the acid. *Trichomonas*, on the other hand, will live for long periods at room-temperature, and therefore, in the above experiments, the acid becomes more potent at a higher temperature.

From these experiments and observations it seems legitimate to conclude that, though the majority of the trichomonads are killed rapidly by $N/20$ HCl or $N/40$ HCl (in excess) a few occasionally can survive for comparatively long periods. I have cultivated these strains of *Trichomonas* for periods of 1 to $2\frac{1}{2}$ years, have examined them frequently, and, for purposes of morphological study, made a large number of preparations. Though I have seen *Trichomonas* in a variety of stages of division and in all stages of degeneration, I have never seen any stage which I could interpret as a cyst or a resistant phase. If there is any morphological distinction determining the greater powers of resistance of a few individuals, it is so small as to be beyond my identification. Also, I have assured myself, experimentally, that the division products of such survivors do not possess any greater power of acid resistance than is possessed by the strain as a whole.

It is to be concluded therefore, that, if *Trichomonas* does not encyst (and, so far, no cysts have been described authentically), and infection does take place from man to man and macaque to macaque—then it is brought about, in individuals who are physiologically normal, by the survival and rapid passage of a few, or even of one *Trichomonas* through the stomach, and its

passage is facilitated, presumably, by an abnormal hypochlorhydric condition of the future host.

The results of these experiments show differences in degree of toleration of the acid among the three strains. Strain *T.* is the most susceptible to the lethal action of the acid—being unable to withstand even 5 minutes' exposure at 37° C., and giving a much larger number of negative results at room-temperature than the *F.T.* or *N.T.* strains. These latter strains were more alike in their susceptibility, though the *N.T.* strain, on the whole, showed a greater degree of acid-resistance. With this strain (*N.T.*) usually more than 15 minutes' exposure to the acid at room-temperature was necessary to kill most of the trichomonads.

Conclusions.

(1) In the foregoing experiments three strains of *Trichomonas* were used: a strain (*F.T.*) of *T. hominis* isolated from human faeces, a strain (*N.T.*) from *Macacus nemestrinus*, and a strain (*T.*) from *M. rhesus*.

(2) All trichomonads of the *T.* strain were killed after 5 minutes' exposure to *N*/20 HCl at 37° C., but positive cultures (1 out of 5 in the *N.T.* strain, and 2 out of 4 in the *F.T.* strain) were obtained from the others.

(3) At room-temperature the survival time of the trichomonads was much longer than at 37° C., a few positive results being obtained up to 60 minutes with *N*/20 HCl, though after 15 minutes' exposure to the acid the proportion of positive results to negatives rapidly diminishes.

(4) The *T.* strain, at both room- and body-temperature, showed the least resistance to hydrochloric acid.

(5) It is to be presumed that when a positive culture is obtained, few trichomonads (or possibly only one) survive the action of the acid. No morphological difference was observed between individual flagellates, in any particular culture, to account for this survival.

(6) The trichomonads cannot withstand drying.

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(*MS. received for publication* 10. x. 1929.—Ed.)

LARVAE OF *CERATOPHYLLUS WICKHAMI* AND OTHER SPECIES OF FLEAS.

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(With 7 Text-figures.)

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I. Introduction.

Having at my disposal a flourishing culture of *Ceratophyllus wickhami*, bred incidentally for another experiment, I made a morphological study of the early stages of this flea, as a basis for the description of other undescribed flea larvae. Although there are many papers which deal with the anatomy of flea larvae, these are rarely of a detailed character, and in the great majority of fleas the early stages are unknown.

In cases where living larvae were available, they were mounted in De Faure's fluid (Imms, 1929). The living larvae were either placed directly into the medium on the slide or previously fixed in 70 per cent. alcohol. Larvae which were fixed on arrival were generally cleared and mounted in Canada balsam, which gave better results than De Faure's fluid when another fixative had already been used.

I am indebted to Dr P. A. Buxton for his advice, and for supplying me with the original *C. wickhami* specimens from a squirrel's nest in his garden; and to Dr K. Jordan for identifying adult fleas for me. My thanks are also due to Prof. G. H. F. Nuttall, F.R.S., for kindly putting at my disposal larvae of

Xenopsylla cheopis, and to Dr L. F. Hirst for the trouble he has taken in sending living *X. astia* from Colombo on many occasions.

II. HISTORICAL.

The earliest description of flea larvae was by Leeuwenhoek (1683). He kept adult fleas in glass dishes, watched them lay eggs, and observed the "worms" hatch and grow until they died on the 12th day. He tried to feed them on flies, but he said that they caused "such a steam on the glass that the worms, being hairy, were entangled in the moisture and remained immovable till they died." He also watched older larvae, which he found fully fed, spinning their cocoons, pupating and emerging as adult fleas.

Cestone (1699) observed fleas through their complete life-cycle and accurately described their habits. He noticed their habit of curling up like a watch-spring, "if they have any fear, they roll themselves up with a brisk and swift motion." He fed the larvae on "the bran-like substance which sticks in the combs when puppies are combed to take out the fleas."

Vallisneri (1733), Roesel (1749) and De Geer (1778) briefly described flea larvae, but were inaccurate in the number of segments to the body, and, where drawings were given, provided their larvae with eyes.

The writers of the nineteenth century (until 1872) produced little new information about flea larvae, and many still repeated the errors of the early authors with regard to the eyes, number of segments and nourishment. Defrance (1824) suggested that the black grains on which the larvae fed were particles of dried blood which flowed from the wounds when the adult fleas sucked the host, and not the faeces as previously thought. But even as recently as 1868, Blanchard wrote praising the maternal instincts of fleas and imagining the mother flea leaving her host to regurgitate recently sucked blood into the mouth of her larva crawling on the ground. It is now known, however, that Defrance had not arrived at the truth of the larval food, as Bacot (1914) has shown that "when the flea is in a gluttonous mood, it discharges, not small grains of uniform size, but drops of red, liquid and scarcely digested blood," and these "on drying assume irregular shapes, little suggesting that they are anal discharges." On this the larvae of many species feed.

Laboulbène (1872) gives a fairly complete account of the "cat-flea" and a figure of the tracheal system with ten spiracles and ventral connectives correctly shown. Künckel (1873) also contributed largely to our knowledge of flea larvae. The authors of the next few years discussed the larval anatomy from the point of view of the affinity of the Aphaniptera to other insects.

Oudemans (1913) gave a very complete account of the larval mouth parts and external anatomy of *Hystrichopsylla talpae* and *Spalacopsylla bisbidentatus* from moles' nests, but he seems to have overlooked one larval instar in his observations, and only seen nine spiracles.

Recent papers of importance are Bacot and Ridewood's (1914) on the external anatomy of larvae of *Pulex irritans*, *Xenopsylla cheopis*, *Ctenocephalus*

canis, *Ceratophyllus fasciatus*, *C. gallinae* and *Leptopsylla musculi*, Perfiljew's (1926) on the internal anatomy of *Leptopsylla pectiniceps* and finally a concise account by Webster (1929) which shows that three species of *Xenopsylla* larvae, *X. cheopis*, *X. astia* and *X. brasiliensis* are practically indistinguishable.

An attempt has been made to give a complete list of references (p. 258) relating to the anatomy of flea larvae besides those cited in the paper.

III. *Ceratophyllus wickhami* Baker.

Host: *Sciurus carolinensis*, the North American Grey Squirrel, now common in this country.

(1) *Breeding.*

The fleas were bred in the laboratory on a Californian grey squirrel. The squirrel was previously combed and given sterilised bedding, before being provided with identified specimens of *C. wickhami*. Samples of adult fleas were taken frequently from the nest and identified to make sure that the culture remained pure. The squirrel's cage was kept on a piece of perforated zinc, with a sheet of shiny black paper under it; below this was a large metal tray. To collect larvae, it was only necessary to sift the smaller particles of material from the nest through the perforated zinc, and the white larvae and eggs showed up clearly on the black paper. For closer observations concerning the growth, ecdyses and length of each instar, the larvae were separated in small glass Petri dishes covered with fine muslin. These were kept in a desiccator over a mixture of sulphuric acid and water in the proportion to give a relative humidity of 80 or 90 per cent. They were kept in a dark cupboard at the temperature of the laboratory. These larvae were fed on dried blood (rabbit or human) ground up finely. Some sand (baked) was also put in the dishes, for use in cocoon making. In the absence of the sand, several larvae pupated without spinning cocoons. If eggs were required of a known age, adult female fleas were taken from the squirrel's nest and put in small glass tubes, each containing a piece of black woollen material, and each covered with fine muslin. The eggs were easy to see and count on the black material. The fleas were left in this for 24 hours, and then returned to the squirrel, a supply of eggs having been laid in the meantime.

(2) *Life-history.*

The eggs are laid singly on leaves or among the smaller particles in the squirrel's nest. They are sometimes found in clusters. They hatch in 8 or 9 days' time. There are three larval instars, the third being composed of an active and a resting period as in other flea larvae. The pupal period varies considerably, lasting 4 days under optimum conditions of temperature and humidity, or several weeks at a less favourable season. An average life-cycle is given in Table I. The conditions for these particular cases were, a relative humidity of 80 per cent., and the laboratory cupboard temperature in June and July 1929 which varied between 16 and 22° C.

(3) *Egg.*

The egg measures 0·50 mm. in length by 0·27 mm. in width. It is oval in shape, and white and glistening in general appearance, but if it is examined under a microscope it can be seen that the chorion is not smooth but minutely pitted all over, giving a granular appearance. The eggs adhere to the substratum on which they are laid.

External anatomy of the larva. A newly hatched larva measures 1·6 mm. in length. The size of a freshly moulted second instar larva varies considerably, the average length being 2·5 mm. When full grown the third instar larva is 4·0 mm. long.

The width of the head capsule was measured in a large number of larvae and gave the following results:

	Average width (mm.)	Minimum width (mm.)	Maximum width (mm.)
1st instar	0·140	0·136	0·143
2nd instar	0·170	0·156	0·186
3rd instar	0·210	0·200	0·214

Table I. *Life of flea in June and July*, 1929.

State of flea	Temp. °C.	Average days	Minimum days	Maximum days
Egg	16–18	7	6	10
1st instar larva	17–20	8	6	11
2nd instar larva ...	20–22	7	3	9
3rd instar larva (active)	18–20	7	6	9
3rd instar larva (resting)	16–18	5	2	8
Pupa	16–18	12	10	18

(4) *Third instar larva.*

The third instar larva is described in detail, whereas the structure of the second and first instar larvae is only given comparatively, and therefore occurs further on in the paper.

The following refers to third instar larvae, before the development of fat, preparatory to pupating.

The larva is composed of a head and thirteen segments (Fig. 4 A). The thoracic segments do not differ in general appearance from the abdominal ones. The tenth and eleventh segments are the widest, the larva tapering from this region to each end. The head and thorax and last three segments are cylindrical, while the intermediate segments are somewhat flattened dorso-ventrally to form a muscular flange on either side. The thirteenth segment bears a pair of anal struts.

The larva is greyish-white and transparent. The head is brown, and the hairs on the body a paler brown, and increase in length from the first segment to the twelfth.

The integument of the body is not smooth but finely sculptured, being covered with minute raised irregular areas, giving a honeycomb appearance.

Head (Fig. 1). The head is more strongly chitinised than the rest of the larva. Viewed from above, it is oval in outline as in other described *Ceratophyllus* (Bacot and Ridewood, 1914) species, and does not taper to any great extent anteriorly, as in the case of *X. astia* and *X. cheopis*. It is very convex

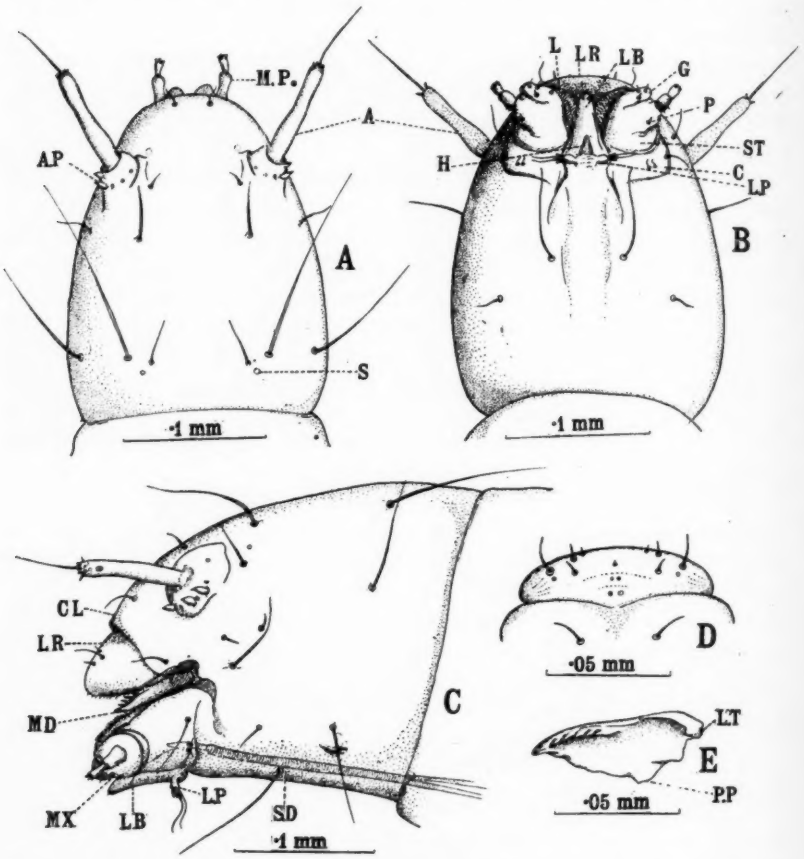

Fig. 1. *C. wickhami*, third instar. A, head, dorsal view: B, head, ventral view: C, head, lateral view: D, labrum, dorsal view: E, mandible, dorsal view. *A*, antennae; *A.P.*, antennal processes; *C*, cardo; *CL*. clypeus; *G*, galea; *H*, hypopharynx; *L*, lacinia; *LB*. labium; *L.P.* labial palp; *LR*. labrum; *L.T*. lateral process of mandible; *MD*. mandible; *M.P.* maxillary palp; *MX*. maxilla; *P*, palpifer; *P.P.* posterior process of mandible; *S*, sensory pit; *S.D.* salivary duct; *ST*. stipes.

dorsally and flattened on the ventral surface. An indistinct suture separates the genae from the epicranium and clypeus. A number of setae of various sizes are regularly arranged on the surface of the head; they are constant in position in all larvae of the same instar. The number, size and positions of these setae

are shown in Fig. 1 A, B and C. There are also a number of circular translucent areas on the head, which on closer examination appear to be shallow pits (Fig. 1 A, *S*). It is possible to make out a very minute hair in the centre of some of these pits. They are probably sensory in function. Although they are always symmetrically arranged, they are not constant in position. The antennae (Fig. 1 A and B, *A*) arise about one-fifth the length of the head from the anterior end and point dorsally and anteriorly. The maxillary palps protrude from the ventral side of the mouth in an anterio-ventral direction. The labial palps (Fig. 1 C, *L.P.*) arise from a median ventral labium (*L.B.*) and are situated the same distance as the antennae from the front of the head.

Antennae. Each antenna is composed of an elongated shaft set in a conical mound. The mound bears six small fleshy protuberances (Fig. 1 A, *A.P.*) on its lateral side. They are cone-shaped and taper to a fine point, corresponding to type B of Bacot and Ridewood's (1914) larvae. Three are larger than the alternating ones. On the inner side of the cone are three sensory pits. Either the pits or tubercles were mistaken for eyes by the early writers (Roesel, 1749, Westwood, 1848 and others). The distal half of the antenna is narrower than the basal part. The extremity is blunt and bears four small spines round the edge and a long median seta. In a few larvae there were two median setae arising from a single socket, one only half the length of the other. There are several small mounds on the end of the antenna between the four spines and a large pit on the external lateral side.

Mouth parts. The labrum (Fig. 1 B and C, *LR.*) is a semi-circular flap, and when the mouth is open it becomes the most anterior part of the head. Its border is smooth and has no median indentation as in *Xenopsylla* and *Ctenocephalus* (Laboulbène, 1872) species. The dorsal surface bears four pairs of setae of various sizes and a minute median one (Fig. 1 D). The external pair are hair-like and curl inwards. The next pair are stout spines and the remaining setae are short and fine. There are also three pairs of sensory pits on the dorsal side. The ventral surface of the labrum is covered with patches of minute spines arranged in three groups. Those of the central group point downwards towards the maxillae, and backwards. Those of the lateral groups all point inwards and slightly backwards. Among these spines are several circular sense pits, similar to those on the head. They are possibly gustatory in function, and belong to the epipharynx.

Mandibles. The mandibles (Fig. 1 C, *MD.* and E) are about 0·07 mm. in length. They lie transversely across the mouth when at rest and their terminal teeth overlap. The distal ends of the mandibles lie in a lower plane than the proximal ends. Each mandible has two points of articulation. The projection (Fig. 1 E, *L.T.*) on the dorso-lateral region of the mandible overlaps a groove in which articulates a projection of thickened chitin, which is part of the clypeus. Another articulation is formed by a posterior projection (Fig. 1 E, *P.P.*) on the mandible and a lower region of the head, probably a groove in the gena, but this is not very clear.

There are six mandibular teeth, a strong terminal one and five dorsal and smaller ones which decrease in size from the point backwards. These point dorsally and inwards when the jaws are at rest, but when in use the teeth point forwards, owing to the fact that the distal ends of the mandibles are in a lower plane than the articulating region. A muscle attached to the lateral projection of the mandible runs back to the inside of the dorso-lateral wall of the head, and is attached to the gena; by its contraction the jaw swings forward. Another muscle, inserted at the posterior projection, extends to the ventral wall of the head at the side of the salivary duct. This, by contracting, would pull the jaw back into its transverse resting position. The mandibles work in a horizontal plane. Their function is primarily to scrape the food and break it up into small palatable particles. But they are also used to help with locomotion. If a larva is watched travelling over a rough surface, such as a piece of cloth, it can be seen to grip a thread with its mandibles and then draw the body forward by contraction of longitudinal muscles, fix itself by placing the anal struts firmly down, and stretch out again to reach another thread.

Maxillae. The maxillae (Fig. 1 C, *MX.*) lie on the ventral part of the head on either side of the labium. They are composed of four incompletely differentiated sclerites and a palp. The posterior sclerite is the cardo (Fig. 1 B, *C*) by which the maxillae are fused to the head. The cardo bears three setae. In front of this is the stipes (Fig. 1 B, *ST.*) separated by a chitinous thickening. The stipes is square in shape if viewed from the ventral aspect. The palpifer (Fig. 1 B, *P*) is fused to the stipes and shows no suture; it bears a two-segmented palp, whose first segment is cylindrical and whose distal segment is short and bears five blunt spines arranged in a circle a little way from the tip. There are two very minute setae on the proximal segment of the palp and a single seta situated on the posterior border of the stipes.

On the anterior border of the stipes is an incompletely separated lobe bearing four setae; this is probably the remnant of a galea (Fig. 1 B, *G*). On the inner and dorsal side of the stipes is another lobe whose surface is covered with numerous spines of equal size. This is the lacinia (Fig. 1 B, *L*). The spines curve towards the central line of the head. In *Hystrichopsylla talpae*, according to Oudemans (1913), the galea and lacinia are fused to form a mala, but in the species described in this paper there are two distinct lobes arising from the stipes anteriorly. It seems reasonable to suppose that among the flea larvae the mouth parts show varying degrees of fusion and represent the beginnings of specialisation in a primitive insect. *Ceratophyllus fasciatus* is an extreme case, in opposition to *Hystrichopsylla talpae* (Oudemans, 1913); it possesses a completely differentiated galea arising from a socket in the stipes, and the cardo in *Xenopsylla* species is even more distinct than in *C. wickhami*, while in some species (Oudemans, 1913) its presence is doubtful.

The maxillae can be extended by being rotated sideways and downwards so that the spines of the lacinia point anteriorly. The rotation is due to the action of two muscles, a longitudinal one from the back of the maxilla to the

junction of head and thorax, and an oblique muscle from the lateral border of the maxilla to the tentorium, which is a thickening of the inner wall of the ventral side of the head and forms a chitinous skeleton for attachment of muscles and for conservation of rigidity in the head. Particles of food, scraped off by the mandibles, are swept into the mouth by the maxillae working on an almost vertical plane but with a lateral or horizontal tendency. The ventral spines of the labrum probably have a similar function. The labium (Fig. 1 B and C, *LB.*) lies between the maxillae. It is broader at the posterior end and bears a pair of labial palps (Fig. 1 B, *L.P.*), each composed of a single segment. Each palp has a pair of blunt processes anteriorly and a pair of long tapering hairs posteriorly placed on the distal end of the segment. Some of the earlier writers thought that the labial palps served for progression, and saw them as a single hooked pseudopod (Bonnet, 1867; Laboulbène, 1872). There are two small setae on the tip of the labium. On its inner side, arising from the floor of the mouth, can be seen the hypopharynx (Fig. 1 B, *H*) with the common duct of the salivary gland opening on it (Fig. 1 C, *S.D.*). The hypopharynx is anterior to the labial palps and lies between the maxillae. Each side of the hypopharynx is bounded by thickened chitin. The vestiges of superlinguae are frequently closely associated with the hypopharynx in the more generalised mouth parts, and Oudemans (1913) describes these structures as the "outer mali of the hypopharynx or maxillulae." Perfiljew (1926) described and figured the mouth parts of *Leptopsylla pectiniceps*. He showed a maxilla with cardo and stipes. He indicated a separate lacinia which he called "inner lobe" and a fused palpifer and galea which he called the "outer lobe" of the stipes.

Body (Figs. 2, 3 and 4). Each body segment, except the last, has a dorsal plate of thickened chitin (Fig. 2 A, *D.P.*), which is almost saddle-shaped. It is not always very distinct, but in larvae which have not recently moulted the saddle shows well. Bacot and Ridewood (1914) also describe small ventral plates round the hairs on the under surface. These are very indistinct in *C. wickhami* and though there appears to be an area of thicker chitin round each ventral seta it has no definite boundary, as in the case of the dorsal plate.

There is a posterior ring of large setae round each segment and another ring of small setae in front of these. The large dorsal setae increase in length from the first to twelfth segment, those of the latter being five times as long as those of the first thoracic segment. The arrangement of these setae is shown in Figs. 2 and 3. The numbers of setae found on each segment are given below; those in brackets represent the number of hairs on the dorsal plate, which may be useful for identification of species (Bacot and Ridewood, 1914).

	Thorax 1–3	Abd. 1–7	Abd. 8	Abd. 9
Large setae	4 (2) pairs	6 (2) pairs	5 (2) pairs	6 (3) pairs
Small setae	6 (2) „	6 (1) „	6 (1) „	6 (1) „

On the dorso-lateral surface of the ninth abdominal segment two of the small setae frequently arise from a common base, but this is not a constant

feature of this species. The tenth or anal abdominal segment has an irregular dorsal comb (Fig. 3 C, *A.C.*) of twenty or twenty-one fine setae. These are arranged in two rows, with usually four anterior and six posterior pairs of setae. There are three pairs of long latero-ventral hairs on this segment and a single pair of small lateral setae.

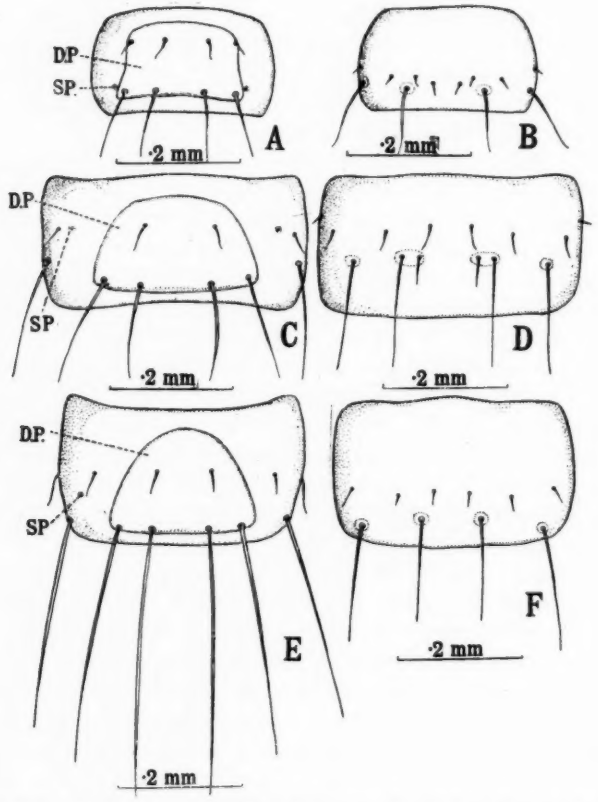

Fig. 2. *C. wickhami*, third instar. A, first thoracic segment, dorsal view; B, ventral view: C, first abdominal segment, dorsal view; D, ventral view: E, eighth abdominal segment, dorsal view; F, ventral view. *D.P.* dorsal plate; *SP.* spiracle.

The anal struts (Fig. 3 C and D, *A.S.*) arise from mounds, each of which bears a row of six short setae on the inner side. The integument of the anal struts and mounds is roughened by numerous excrescences. This sculpturing of the chitin is much coarser on this segment than on the rest of the body. There is a region of thick chitin on the dorsal surface of the anal struts. The struts, as seen laterally, are thick, and only slightly curved and tapering to a blunt end.

The spiracles (Fig. 2 A, C and E, *SP.*) occur on the first and third thoracic segments and the first eight abdominal segments. The tracheal system (Fig. 4 A) is composed of a double longitudinal trunk running down each side of the body, joined in the second to eleventh segments by short vertical tracheae.

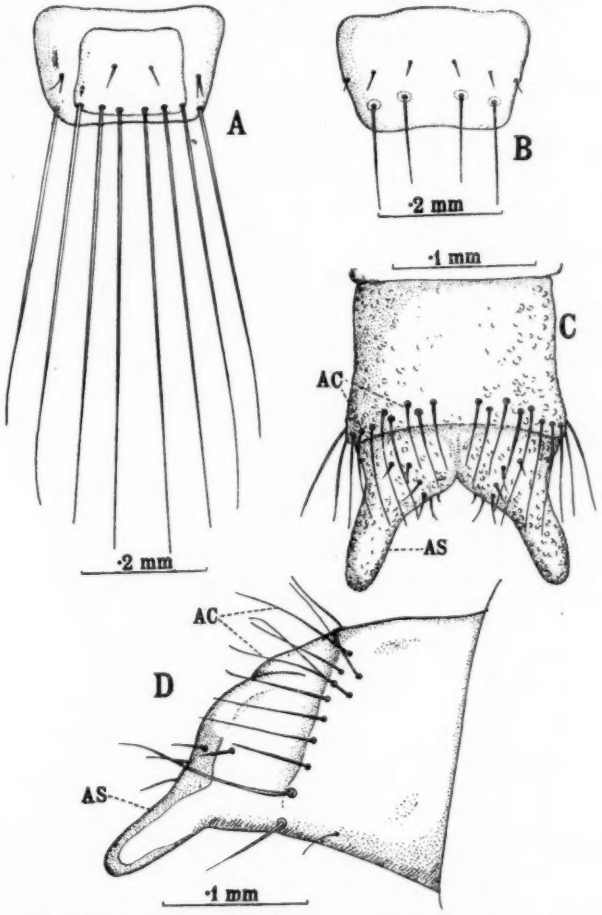

Fig. 3. *C. wickhami*, third instar. A, ninth abdominal segment, dorsal view; B, ventral view: C, tenth abdominal segment, dorsal view; D, lateral view. *A.C.* comb on anal segment; *A.S.* anal struts.

In the first thoracic segment the two main trunks of a side join and give off branches to the head. The first thoracic spiracle is in the posterior part of the segment almost in a line with the large posterior setae and lateral to the dorsal plate. The spiracle on the third thoracic segment is in the anterior half of the segment and in front of the row of small setae. The abdominal spiracles of

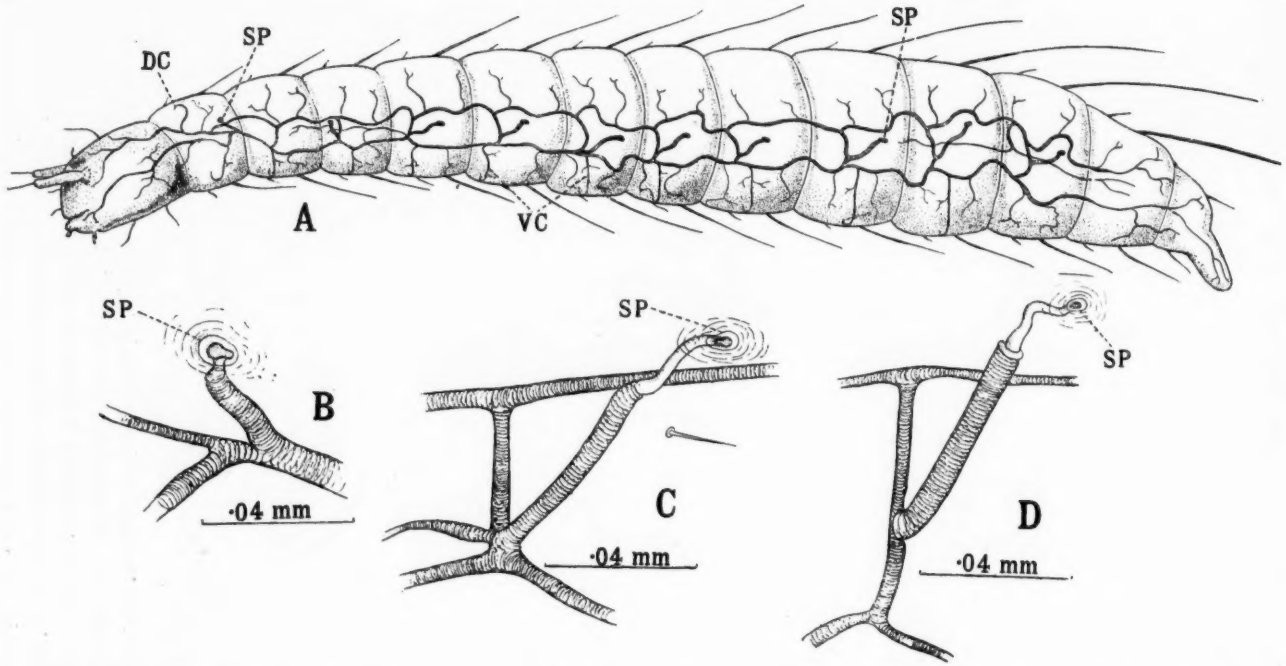

Fig. 4. *C. wickhami*, third instar. A, tracheal system of left side: B, spiracle and tracheae of first thoracic segment: C, spiracle and tracheae of first abdominal segment: D, spiracle and tracheae of eighth abdominal segment. *D.C.* dorsal connective between lateral tracheal trunks; *SP.* spiracle; *V.C.* ventral connectives between lateral tracheal trunks.

the first to the seventh segments lie between the small anterior setae at the edge of the dorsal plate. Those of the eighth segment are situated in the posterior half of the segment between the rows of large and small setae. Each spiracle communicates with a short trachea which arises from the vertical connective of its segment between the longitudinal trunks, except the first thoracic spiracle whose spiracular trachea is a branch from the lower trunk. The spiracular tracheae of the thoracic segments run directly to the surface, but those of the abdominal segments run backwards for a short distance before opening on the surface.

The thoracic spiracles (Fig. 4 B, *SP.*) are keyhole-shaped, and are larger than the abdominal ones (Fig. 4 C and D, *SP.*) which are pear-shaped. The spiracles are raised slightly above the integument and are surrounded by an area of soft, wrinkled chitin.

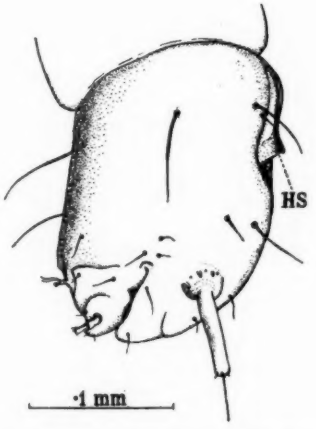

Fig. 5. *C. wickhami.* Head of first instar larva, lateral view. *H.S.* hatching spine.

The main tracheal trunks of the two sides are united below by ten ventral connectives (Fig. 4 A, *V.C.*) in the thoracic and first seven abdominal segments. There is also a dorsal connective trachea (Fig. 4 A, *D.C.*) in the first thoracic segment.

The pair of spiracles in the third thoracic segment seems to have been overlooked by many recent writers, although Laboulbène observed and figured it in 1872.

(5) *Second instar larva.*

The chaetotaxy and general appearance of the second instar larva appear to be identical with that of the third instar larva, the two stages differing only in size and the fact that the setae on the body are paler brown than in the final stage.

(6) *First instar larva.*

A first instar larva may be recognised by the egg-burster or hatching spine (Fig. 5, *H.S.*) which is situated far back in the dorsal region of the head. It is a small chitinous tooth lying in a depression of the epicranium. It is part of the first instar skin in fleas and is therefore lost after the first moult. In most other insects in which an egg-burster occurs, it is on "a chitinous enveloping skin which is shed at hatching," as shown by Gimingham (1926) for the Aphidae. The hatching spine in flea larvae was first noticed by De Geer in 1778, but its function was not observed until Künckel (1873) wrote about it a hundred years later.

The setae on the body of the first instar larva are similar in number and position to those of older instars. Both the large and small rings of setae are present on each segment. The setae are only slightly darker than the body.

The larva is cylindrical and of almost uniform diameter, with no sign of the lateral muscular flange seen in later instars.

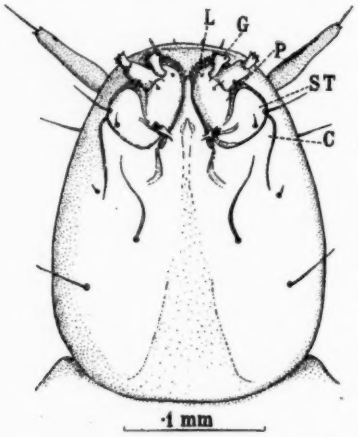

Fig. 6. *C. fasciatus*, third instar. Head, ventral view. *C*, cardo; *G*, galea; *L*, lacinia; *P*, palpifer; *ST*. stipes.

IV. *Ceratophyllus fasciatus* Bosc.

Only full-grown larvae of *Ceratophyllus fasciatus* have been examined. Bacot and Ridewood's (1914) description of this species has been confirmed, but it seems necessary to add to this a figure of the ventral side of the head.

The maxillae are composed of the same parts as in *C. wickhami*. The cardo (Fig. 6, *C*) is somewhat reduced, but the sclerites of the stipes (Fig. 6, *ST.*) are better developed than in other described species. The lateral sclerite or palpifer (Fig. 6, *P*) is partly differentiated by a thick ridge of chitin. The two distal lobes are quite distinct. The galea (Fig. 6, *G*) is triangular and arises from a simple socket in the stipes. The lacinia (Fig. 6, *L*) is a larger lobe further

back on the dorsal side of the stipes, and protruding into the mouth. It is covered with spines on its inner border.

There are two setae on the posterior border of the stipes, and a small one on the anterior border. The palpifer bears one seta and there is also a single one on the proximal segment of the maxillary palp. The galea has a pair of terminal setae.

The labium is similar to *C. wickhami* but increases in width posteriorly so that the labial palps lie further apart.

The tracheal system is the same as in the previous description, with ten spiracles.

V. *XENOPSYLLA CHEOPIS* Roths.

Larvae of *Xenopsylla cheopis*, representing progressive growth stages from the 1st to the 17th day, were sent to me by Prof. G. H. F. Nuttall. These he had obtained from Captain G. Liston, I.M.S., Bacteriological Laboratory, Parel, India, in 1909. Judging from the length of the heads of these larvae, it appeared that those of the first 5 days were first instar, and the remainder were second instar. This assumption was based on the head measurements given by Bacot (1914) for *X. cheopis*.

1st instar	...	0·14 mm.
2nd instar	...	0·16 mm.
3rd instar	...	0·20 mm.

Most of the observations by Bacot and Ridewood (1914) and by Henderson (1928) on the growth and external characters of the larvae were confirmed. But in certain respects the larvae seemed to differ. In addition, further details of the head and mouth parts are figured below.

The larva bears an egg-burster (Fig. 7 B, *H.S.*) on the epicranium until the 5th day, when the first ecdysis takes place.

Head. The setae on the dorsal and lateral sides of the head are figured by Bacot and Ridewood (1914). A figure is given below of a ventral view of the head to show the chaetotaxy and structure of the mouth parts (Fig. 7 A). In all larvae examined the antennae appeared to be composed of a single joint inserted in a flattened dome bearing three blunt processes alternating with three smaller ones. Henderson indicated two segments to the antenna and only five blunt processes on the mound. A pair of sensory pits were present near the distal end of the antenna; the pit on the inner side being smaller than the outer one. A small blunt process was also present between the sensory pits, as described by Henderson (1928).

The labrum has a cleft in the middle. It has five pairs of setae on the dorsal surface, arranged as in Fig. 7 C. Three of these are stiff and spine-like, and the other two pairs are minute hairs. A translucent sensory pit, similar to those described for *C. wickhami*, is situated behind the labral cleft. The ventral surface of the labrum is covered with fine setae arranged in three groups, with a few sensory pits among them.

The mandibles (Fig. 7 D) of second instar larvae are 0·06 mm. in length. They have five teeth which are directed dorso-anteriorly when at rest. The lateral projection, where the exterior muscle is attached, is more prominent than in *C. wickhami*.

The four parts of the maxillae, mentioned above, are visible in *X. cheopis*. The cardo (Fig. 7, A, *C*) has a pair of long setae on its posterior border. A two-segmented palp (Fig. 7 A, *M.P.*) is situated on the stipes; at its extremity, five blunt processes are arranged in a circle. On the proximal segment of the palp, two minute setae are situated close together on the posterior side. The

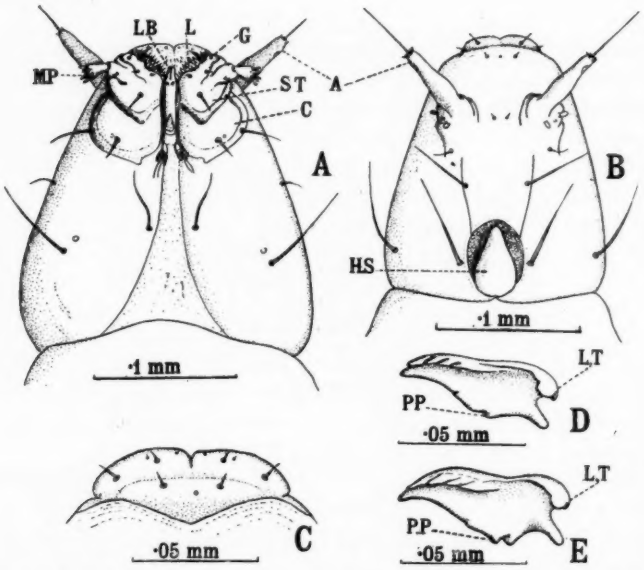

Fig. 7. *X. cheopis* A, B, C, D; *X. astia* E. A, head, second instar, ventral view: B, head, first instar, dorsal view: C, labrum, second instar, dorsal view: D, mandible, second instar, dorsal view: E, mandible, third instar, dorsal view. *A*, antennae; *C*, cardo; *G*, galea; *H.S.* hatching spine; *L*, lacinia; *LB.* labium; *L.T.* lateral process; *M.P.* maxillary palp; *P.P.* posterior process; *ST.* stipes.

galea (Fig. 7 A, *G*) is represented by an incompletely separated lobe bearing three setae ventrally and a strong spine on the anterior border. The lacinia (Fig. 7 A, *L*) is covered with small teeth which point towards the labrum.

The labium (Fig. 7 A, *LB.*) is bifid, and has two small finger-like processes near the distal end. It bears the labial palps, which are triangular lobes with four processes similar to *C. wickhami*. The hypopharynx is visible lying on the floor of the mouth, just in front of the labial palps.

Body. The segments of the body increase in size from the first to ninth segment, and then decrease abruptly to the anal struts. There are ten spiracles

arranged as in *C. wickhami*, and a similar tracheal system. Webster (1929) says that *X. cheopis*, *X. astia*, and *X. brasiliensis* all have ten spiracles.

The setae are arranged in two rows round each segment in both first and second instar larvae. The large posterior hairs agree with the numbers given by Bacot and Ridewood (1914). In first instar larvae the small anterior hairs on the thoracic segments, though small, are constantly present. Henderson (1928) omitted these from first instar larvae.

	Thoracic 1–3	Abd. 1–6	Abd. 7 and 8	Abd. 9
Large setae	5 (2) pairs	5 (1) pairs	6 (2) pairs	7 (3) pairs
Small setae	6 (2) „	5 (1) „	6 (2) „	5 (1) „

These figures might equally well stand for *X. astia*, as the number and position of setae are the same. The figures in brackets refer to the number of setae on the dorsal plate of each segment. The tenth abdominal segment is described by Henderson (1928). The anal comb consists of twenty-two or twenty-four fine curved setae, with three pairs of thick ventro-lateral setae. There is a double row of about eight setae on each anal mound, with usually three irregular setae near the dorsal part of the mound.

VI. *XENOPSYLLA ASTIA* Roths.

On several occasions in May and June living *Xenopsylla astia* were sent by Dr L. F. Hirst from Colombo. They travelled in tubes containing dried blood and bran. On arrival the larvae had reached the third instar, and a few were pupating.

Only third instar larvae have been examined.

X. astia measures 4·2 mm. in length when full grown. The measurements given in this paper refer to extended specimens, previously fixed in 70 per cent. alcohol. The head length varies between 0·18 mm. and 0·20 mm. The larva is slightly smaller than that of *X. cheopis*, taking Bacot and Ridewood's (1914) measurements for the latter.

All the larvae examined appear to be indistinguishable from *X. cheopis*, except for slight differences in the mandibles and antennae. The setae are similar in number and position on all parts of the body, head and mouth parts.

The antennae are comparatively shorter than those of *X. cheopis* and in all available specimens only one lateral pit was visible. Three larger processes alternating with three smaller processes were always present on the antennal mound.

The mandibles (Fig. 7 E) have five teeth as in *X. cheopis*. A difference in shape between the mandibles of these two species of *Xenopsylla* is seen in the region where the retractor muscle is inserted (Fig. 7 D and E, *P.P.*). There are two very prominent projections at this point in *X. astia*, whereas *X. cheopis* only has two small lobes which do not protrude to any noticeable extent. A wider mandible and a deeper depression between the posterior (Fig. 7 D and E, *P.P.*) and lateral projections (Fig. 7 D and E, *L.T.*) is thus formed in

X. astia. In cleared specimens of the larvae mounted with the dorsal side uppermost, *X. astia* and *X. cheopis* larvae can be distinguished by this character, although Webster (1929) stated that the two species are indistinguishable. Unfortunately this comparison is between mandibles of second instar *X. cheopis* and third instar *X. astia*, but judging from other flea larvae, there seem to be little or no differences in the morphology of the three instars, and therefore it seems reasonable to suppose that the mandible of a species of flea will be the same shape in all three stages.

Summary.

A short historical account is given of the writings on flea larvae since the seventeenth century. The larvae have been known since Leeuwenhoek tried to breed them in 1683.

The life-history of *Ceratophyllus wickhami* is described. The fleas were bred in the laboratory on a grey squirrel. Three larval instars occur, and the life-history occupies, on an average, 6 weeks under experimental conditions.

The external anatomy of the third instar larva of *C. wickhami* is described. The mouth parts are generalised and suggest the condition of a primitive insect. The tracheal system is composed of a double longitudinal trunk on each side, with spiracles on the prothorax, metathorax and first eight abdominal segments.

The first and second instar of *C. wickhami* are similar to the third instar larvae, except in size and the presence of a hatching spine in the first stage.

The larva of *Ceratophyllus fasciatus* is mentioned. The importance of the species lies in the presence of completely separated galea and lacinia.

Larvae of *Xenopsylla cheopis* and *X. astia* are briefly described. The larvae of the two species are practically identical except for the shape of the mandibles.

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(*MS. received for publication* 27. XI. 1929.—Ed.)

NATURAL TRANSMISSION OF HEART-WATER OF SHEEP BY *AMBLYOMMA VARIEGATUM* (FABRICIUS 1794).

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(With Plate XXX and 3 Charts.)

The disease "Heart-water" of sheep, cattle and goats has been recognised in South Africa for upwards of 70 years, and as long ago as 1902 Lounsbury demonstrated the part played by the common bont-tick, *Amblyomma hebraeum* Koch 1844, in its transmission. The virus of the disease does not pass through the egg of the tick, but a tick infected in the larval or the nymphal stages is capable of transmitting the infection when feeding as a nymph or adult respectively. Further, according to Lounsbury, a tick infected as a larva may feed upon an insusceptible host during the nymphal stage, and still remain infective until the final feed as an adult. According to the South African workers the period of incubation is about 14 days when induced by tick-infestation, and somewhat shorter when direct blood inoculation is employed. The onset of the disease is characterised by a severe febrile reaction, which may or may not be accompanied by early manifestations of hydrothorax and hydropericardium. Before death nervous symptoms usually develop, *e.g.* muscular tremors, strabismus, and tetanic convulsions. The nervous phenomena are much more pronounced in cases of the disease in cattle, and in fact together with the pyrexia may be considered as the typical syndrome of the disease in this species.

At death the characteristic lesion, hydropericardium, is present in only a percentage of animals and is much less frequently observed in cattle than in sheep or goats. Other lesions found are those of an acute septicaemia; *tumor splenis*; petechial haemorrhages in the epicardium, myocardium, endocardium, and cortex of the kidney; gastritis; and injection of the blood vessels of the cerebral meninges.

Recently Cowdry (1925) has described the occurrence of a *Rickettsia* (*R. ruminantium*) in the endothelial cells of the smaller blood vessels of the kidney, cerebral cortex, medulla, cerebellum, spleen, pancreas, suprarenals, lymphatic and salivary glands, ovary, and *corpus luteum* in animals that have died from experimental heart-water (induced either by blood inoculation or by infestation with infected ticks), or that have been killed during the febrile reaction.

The organisms are gram-negative, intracellular and coccoform, occurring characteristically in spheroid or ovoid masses in the cytoplasm of the cells. Several clumps of *Rickettsiae* are commonly present in a single cell, but apart from distortion of the cell or its nucleus, when the masses are large, their presence appears to have no deleterious effects on the parasitised cell. Certain histological changes are regularly found in such organs as brain, kidney, and liver, but these lesions, as Steck (1928) has pointed out, are in no way correlated with the presence of *Rickettsia* in the area where the lesions are observed or even with their existence, as far as can be ascertained by histological examination, in the whole organ. The lesions cannot therefore be considered as a reaction to the invasion of endothelial cells by *Rickettsia*.

Amblyomma hebraeum, the vecting tick in South Africa, has not been recorded from Kenya Colony, although the disease heart-water is widely distributed throughout the Highlands, and particular attention has been directed to a search for this species since the arrival of the writer in 1925. According to Robinson (Nuttall, Warburton and Robinson, 1926) the centre of distribution for this species is South Africa, particularly the south-eastern regions, extending northwards through Portuguese East Africa to Tanganyika Territory.

Two other species of *Amblyomma*, neither of which occurs in South Africa proper, have their centre of distribution in Tropical Africa: *Amblyomma gemma* Dönitz 1909, which extends from Somaliland in the north through Eastern Africa to Tanganyika Territory in the south; and *A. variegatum* (Fab. 1794), which ranges from Egypt and Abyssinia in the north to Portuguese East Africa in the south, occurring also on the west coast.

Morphologically *A. gemma* resembles *A. hebraeum* more closely than any other species, and it has not infrequently been collected by the writer from cattle, sheep and goats, on heart-water-infected farms, usually in rather small numbers and invariably mixed with *A. variegatum*, which is a much more common species in the same districts. While it is regarded as a possible vector of heart-water in Kenya Colony, it is considered that its comparative rarity relegates it to a position of only secondary importance, and for the same reason an opportunity has not yet arisen to carry out transmission experiments.

A. variegatum is by far the commonest member of the genus in Kenya and has been suspected of transmitting heart-water ever since the disease was recognised in this country. It is, however, an extremely difficult species to rear in the laboratory, and in consequence definite proof has been difficult to obtain. Lounsbury (1905) states that the eggs take the same time to hatch and the larvae and nymphae the same time to feed and moult as would those of *A. hebraeum* under the same conditions, but Nuttall (1915) was able to observe hatching of eggs in only two batches which were incubated at 30° C. and all attempts to raise the larvae failed, although he was able to rear generations of *A. hebraeum* with ease. The experience of the writer accords

with that of Nuttall, and up to the present *A. variegatum* has resisted all attempts at rearing in this laboratory.

The occurrence of three cases of naturally contracted heart-water in a small fenced enclosure at the laboratory recently afforded an opportunity to demonstrate the natural transmission of heart-water by *A. variegatum*. The history of this small outbreak is as follows:

Two cattle, Nos. X 3386 and X 3245, that had been used as virus-makers during double inoculations against rinderpest in the field, were returned to the laboratory on March 4th, 1929, and segregated in a small fenced paddock for some days. X 3386 died on March 5th, apparently from the sequelae of rinderpest; and X 3245 died on March 18th, 1929, from East Coast Fever.

On June 27th, 1929, a privately owned sheep that had been for some days in this enclosure was found dead. The lesions discovered at autopsy suggested heart-water as the cause of death, and by histological examination *Rickettsiae* were demonstrated in the capillaries of the brain and kidney. Four male specimens and one female of *A. variegatum* were removed from this sheep after death. Four days later, on July 1st, a laboratory sheep, No. 3077, in the same enclosure was found dead, and again the gross lesions found at autopsy indicated heart-water as the cause of death. Histological examination confirmed the diagnosis by demonstrating large numbers of *Rickettsiae* in the brain and kidney. One male specimen only of *A. variegatum* was collected from this sheep. Sub-inoculations of heart-blood of Sheep 3077 intravenously into two other sheep, produced no reactions. One month later, on August 5th, Cow 6074, which had been in the same enclosure for several weeks, died of heart-water; again the diagnosis was confirmed by the examination of sections of brain and kidney. This cow had nine male and four female *A. variegatum* on her at death; the females were all attached and one was almost fully engorged. Sub-inoculations of the heart-blood of Cow 6074 were made into two sheep, Nos. 3225 and 3068. Sheep 3068 gave an indeterminate reaction during the third and fourth weeks after inoculation. Sheep 3225 died unexpectedly on the 17th day without having shown any febrile reaction. There were no lesions suggestive of heart-water, and neither *Rickettsia* nor lesions could be demonstrated by histological methods. Of three sheep inoculated with heart-blood of Sheep 3225 none showed any reaction.

As no cases of heart-water had occurred elsewhere on the laboratory farms, and *A. variegatum* cannot normally be collected from the laboratory animals, it was concluded that the cattle X 3386 and X 3245 had in all probability been responsible for the infestation of this enclosure by dropping engorged nymphae. No other animals from *Amblyomma*-infested districts had recently been kept in this small paddock. The possibility that one or both of these animals was infected with heart-water at the time of death cannot be excluded, since histological examinations for *Rickettsia* or heart-water lesions were not carried out. It is interesting to note the time elapsing between the arrival of these animals and the occurrence of the first case of heart-water, 125 days, of which

one month was extremely rainy. This presumably is the time required by nymphae to moult, feed and produce infection. Allowing roughly 25 days for the feeding and incubation periods the nymphae apparently took some 3 months to complete the moult.

EXPERIMENTAL INFECTION BY TICKS (*A. variegatum*).

Unfed adult ticks were next collected by placing calves in the enclosure and removing any attached ticks once or twice daily. The supply seemed now almost exhausted since it was only possible to collect two females and ten males over a period of 2 weeks, and no more have been observed since that time. No larvae or nymphae of *Amblyomma* were observed at any time on any of the animals placed in the paddock after the death of the original two cattle, although a special search for the immature stages was made in every case.

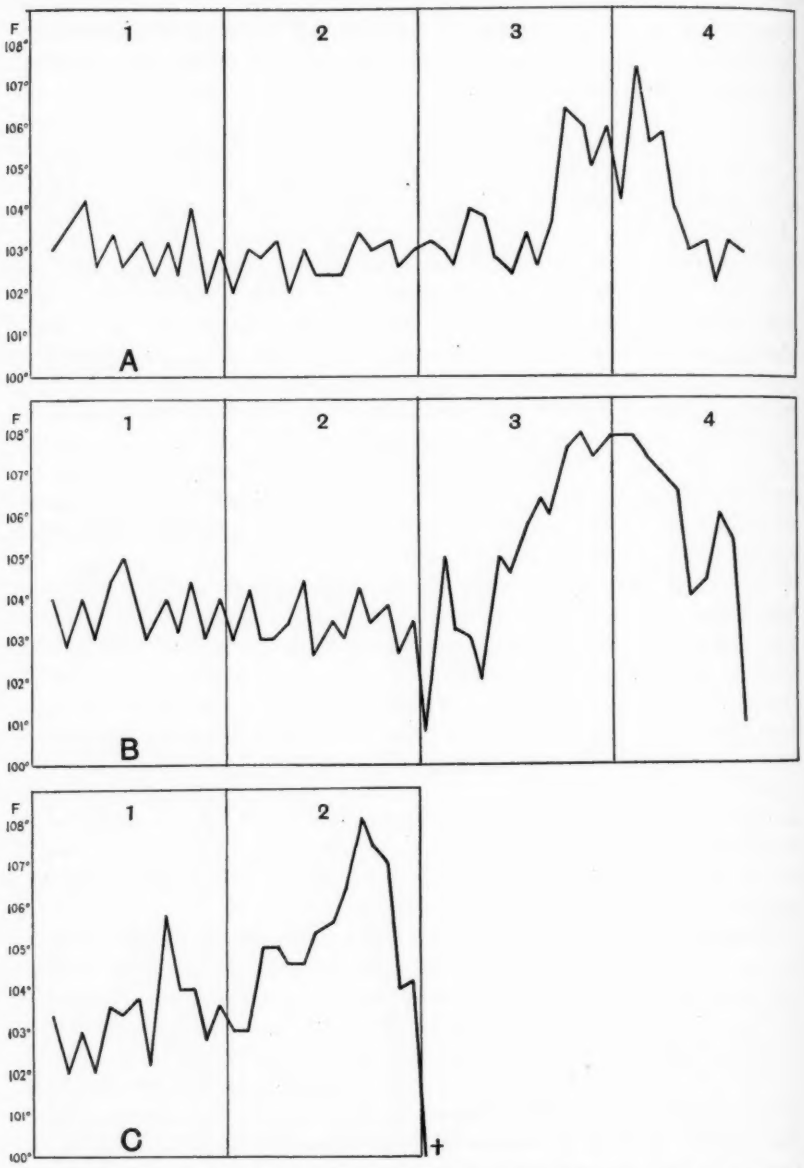
Sheep A and B (3133 and 3129) were each infested on August 7th, 1929, with four unfed specimens of *A. variegatum* (3 ♂, 1 ♀) collected from calves in the enclosure. Both sheep reacted on the 20th day. The febrile reaction persisted for 4 days in Sheep A. In Sheep B the reaction was more severe, the temperature for 4 days remaining above 107°; altogether the reaction of this animal lasted for 16 days. The Charts of both animals A and B are appended. Both sheep recovered, having displayed no other objective symptoms.

Blood was taken from Sheep B on the afternoon of August 28th, temperature of the animal 106°, and injected intravenously in amounts of 10 c.c. each into Sheep C, D and E, 3233, 3219 and 3240. All three animals reacted. Sheep C showed the commencement of a reaction on the 9th day and died on the 15th day: Sheep D reacted on the 12th day and was destroyed on the 14th day: Sheep E reacted on the 12th day and died on the 20th day. The temperature record of C is shown in the Chart.

Rickettsiae were demonstrated in sections of the superficial cerebral cortex and hippocampus of Sheep D, destroyed 36 hours after the febrile reaction had commenced. The characteristic lesions of leucostasis, infiltration of the pia mater, perivascular infiltration in the cortex and attraction of satellite cells by neurons were present but not marked.

Sheep C showed large numbers of *Rickettsiae* in sections of the cerebral cortex and kidney. Leucostasis and perivascular infiltration were marked, but there was also an enormous infiltration into the sub-arachnoid space, and in this exudate and also in the cells bacteria were found. On this account the lesions in this sheep were excluded from further consideration.

Sheep E survived for a period of 8 days from the commencement of the pyrexia, and showed the characteristic gross lesions of heart-water at autopsy. Histological methods showed that *Rickettsiae* were extremely rare in the cerebral cortex, over an hour's search being necessary before a single group could be found. None were found in sections of kidney. Nevertheless the usual microscopic lesions were exceptionally well marked, and it may be assumed that the severity of the changes, particularly in the central nervous system is



A and B. Sheep 3133 and 3129, infected with heart-water by *Amblyomma variegatum*. Both animals recovered.

C. Sheep 3233, infected by inoculation with blood of Sheep 3129. Died on fifteenth day.

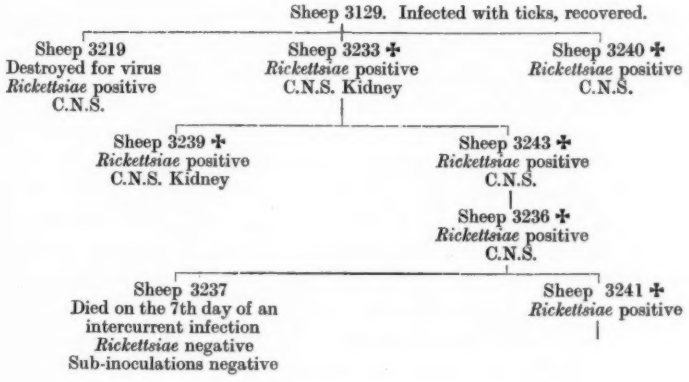
related to the longer duration of the infection. Sections of kidney showed marked perivascular infiltration in the boundary zones, and some periglomerular cellulation. In the cerebral cortex there was marked leucostasis, perivascular infiltration and satellitism of monocytes around the neurons.

Blood from Sheep C was injected intravenously into Sheep 3239 and 3243. Both these animals died on the 15th day after inoculation and both showed lesions and *Rickettsiae*. From Sheep 3243 a further sub-inoculation was made into Sheep 3236, which died on the 13th day after inoculation, and again *Rickettsiae* were demonstrated, together with infiltrative lesions of the kidney and central nervous system.

Two sheep, Nos. 3237 and 3241, were inoculated with blood from Sheep 3236. Sheep 3237 died on the 7th day after inoculation from an intercurrent infection. Examination of the kidney and central nervous system for *Rickettsiae* or lesions gave a negative result, as did also the sub-inoculation of heart-blood into two susceptible sheep. Sheep 3241 died on the 19th day after inoculation after a reaction lasting 7 days.

The strain is still being propagated for experimental purposes by blood inoculations in sheep, and shows no signs of decreasing in virulence.

The history of this strain of virus for the first four generations is shown in diagrammatic form in the following table.



DISCUSSION.

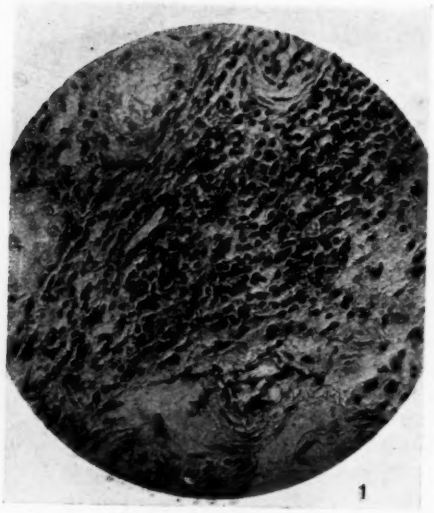
The observations here recorded show that heart-water of ruminants in Kenya Colony may be transmitted naturally by the adult stages of *Amblyomma variegatum*. The prepatent period after infestation with infected ticks is about 3 weeks; after direct blood-inoculation from a reacting animal the period of incubation is usually from 9 to 12 days. The findings of Cowdry (1925) with reference to the regular occurrence of *Rickettsiae* in experimentally infected animals are confirmed, and the *Rickettsiae* demonstrated in these animals (Pl. XXX, figs. 6 and 7) agree morphologically

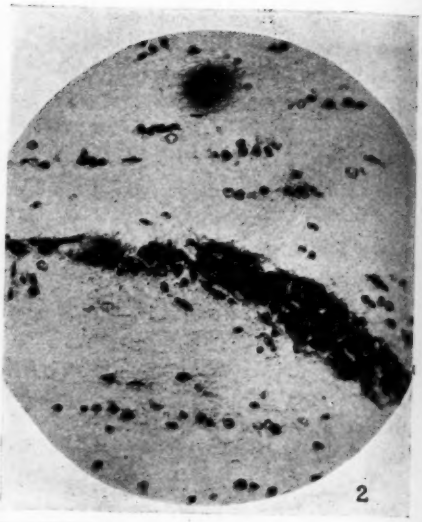
with *R. ruminantium* Cowdry. In their uniformity of shape and size these coccoform organisms are strikingly different from the other *Rickettsiae* that have been described as pathogenic for mammals.

For some 3 years routine histological examinations of the kidney and central nervous system have been carried out at this laboratory whenever heart-water was suspected as the cause of mortality in stock, and undoubtedly the search for *Rickettsiae* has enabled the staff to diagnose outbreaks of the disease that would have remained undetected by naked-eye methods and frequently unconfirmed by animal inoculations. In the course of these routine examinations certain lesions, particularly of the central nervous system, were remarked as characteristic of the disease, and it was later noticed that such nervous lesions were usually more marked in the bovine cases, a fact which agrees well with our knowledge of the symptomatology of the disease in cattle. Reference has already been made to these lesions in the text of this paper, and Steck (1928) sums up his account of the lesions in the following terms: "The characteristic changes in heart-water are leucostasis and perivascular cellulation. The former occurs in all organs and the macrophage is the prominent cell, but lymphocytes and neutrophiles are also numerous. The latter is pronounced in the liver, kidney, and sometimes the adrenal glands. All the cells of the mesenchyma may take part in this reaction, but mainly those of the lymphocytic series: lymphocytes and plasma cells. It seems most likely that these alterations are due to a noxe which is spread diffusely by the blood-stream. It must remain for further investigation to determine the nature of this noxe."

It is not proposed to discuss the lesions in detail in this paper, but merely to state that the findings of Steck have been substantiated in course of examination of sections from a large number of naturally occurring cases of heart-water, and from experimentally produced cases of the disease. The perivascular cellulation is usually most marked in the boundary zone of the cortex of the kidney (Pl. XXX, fig. 1). Leucostasis is the early characteristic change observed in sections of cerebral or cerebellar cortex. It is chiefly remarked in vessels a little larger than those in which it is customary to find *Rickettsiae*, and when combined with the perivascular infiltration in the kidney it is regarded by the writer as of considerable assistance in diagnosing those cases of heart-water in which *Rickettsiae* cannot be demonstrated. In addition to leucostasis some evidence of perivascular infiltration can usually be found in the cerebral cortex (Pl. XXX, figs. 2 and 3), and occasionally there is a certain amount of cellular exudate into the subarachnoid space.

A lesion which has not hitherto been described, but which is present with great regularity in cases of the disease in bovines, and frequently also in sheep when they have survived several days after the commencement of the reaction, is the so-called "Satellitism" of neurons (Pl. XXX, figs. 4 and 5). The condition is in every way comparable to that described by various authors (Nicolau and Galloway, 1928, and others) in Borna disease of the horse, and by Metchnikoff in senility. In Borna disease it is stated that the condition may either dis-





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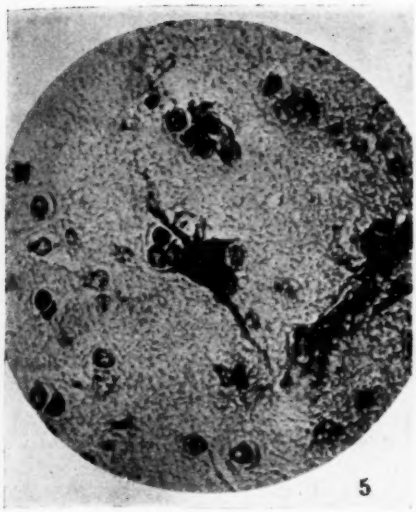

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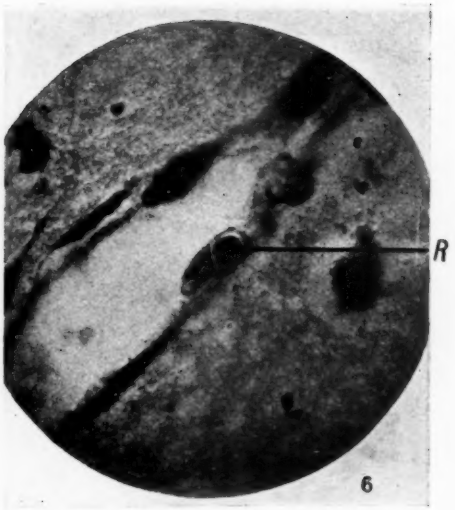

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appear during the evolution of the disease, or become intensified and lead to neuronophagia. The writer has observed occasional evidence of what appears to be true neuronophagia in cases of heart-water in cattle, but the phenomenon is rare. It may be that the "Satellitism" in heart-water is merely a result of injury of the neurons by some toxin derived from the *Rickettsia*, but here it will be remarked as curious that in the immediate vicinity of the demonstrable *Rickettsiae* there should be no evidence at all of any injury to the host-cells or of reaction to the presence of the organism. On the other hand it is quite possible that the *Rickettsia* infection succeeds in "lighting-up" a widespread but normally latent neuro-virus, which under these conditions is capable of giving rise to the neuron lesions, and also to the nervous symptoms commonly associated with heart-water. Much the same explanation has been offered in connection with the peculiar incidence of such diseases as herpes, post-vaccinal encephalitis, etc. Further investigations, however, are necessary before the true explanation of these nerve-lesions can be established.

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EXPLANATION OF PLATE XXX.

Experimental Heart-water in Sheep.

Fig. 1. Perivascular infiltration in kidney of Sheep 3233. (× 250)

Fig. 2. Leucostasis in vessel of cerebral cortex of Sheep 3233. (× 250)

Fig. 3. Early perivascular infiltration in cerebral cortex of Sheep 5290, natural case of heart-water. (× 90)

Figs. 4 and 5. "Satellitism." Neurons in cerebral cortex of Sheep 3240, experimental infection. (× 490)

Fig. 6. Clump of *Rickettsia* (*R*) in endothelial cell of capillary in the cerebral cortex of Sheep 3014, natural case of heart-water. (× 1100)

Fig. 7. Mass of *Rickettsia* (*R*) in endothelial cell of glomerulus in kidney of Sheep 3239, experimental infection. (× 1100)

(*MS. received for publication* 2. XII. 1929.—Ed.)

AN ANALYSIS OF THE METHODS USED IN THE STUDY OF LARVAL TREMATODES.

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Much of the confusion and difference of opinion concerning larval trematodes is due to incomplete and imperfect descriptions of the species. The use of fixed and stained material frequently gives a very distorted idea of the form and structure of these minute and active worms. In such soft-bodied animals, devoid of skeletal structures, the extension and retraction of different portions of the body cause much variation in shape and in relative position of the several parts. Often extreme, abnormal, or grotesque shapes are assumed when the specimens are placed in killing fluids. With certain reagents the larvae are swollen, whereas with others they are much contracted. Obviously, the study of such material cannot give a true and complete concept of a species.

The study of living specimens, on the other hand, shows the extent of form-changes which normally occur during locomotion or in the extension and retraction of different regions. Frequently, more of anatomical and histological detail can be distinguished in unstained, flattened, moribund specimens than in the best fixed and stained material. The advantages of the study of living trematodes were clearly predicated by Looss (1894). As noted by Looss and confirmed by later investigators, the excretory system can best be observed in living specimens. With a sufficiently high incidence of infestation to provide specimens for repeated observations, it is possible to work out the morphology of a species from the study of live material alone. Frequently, however, hundreds of snails must be examined to find a particular species, and scarcity of material necessitates the completion of a description from preserved specimens. Although each method supplements the other, the use of living material can be made to yield data of far more value and significance than can be secured from fixed specimens, studied either entire or in sections.

As observed by several investigators and noted by Brumpt (1929) cercariae commonly emerge from the sporocysts or rediae in which they are formed while yet immature, and complete their development in the lymph spaces of their molluscan hosts. According to my own observations, the cercariae of *Cryptocotyle lingua* may leave the rediae before the development of the cuticular spines, the formation of the membranous keel of the tail, and before the gland cells become filled with secretory products. When infected snails are crushed, the larval trematodes are freed, but the cercariae are usually

immature and frequently differ distinctly, even in morphological features, from those which emerge normally. Many published descriptions of cercariae are based on the study of such immature specimens, and consequently the observations, although accurate for that stage of development, are misleading.

Moreover, if the host is killed, the trematode larvae do not long survive, and in cases of rare or uncommon species the loss of the infected snail is serious. If snails are isolated in individual dishes, it is possible to recognise those with mature infections, since cercariae pass out into the water in the course of 24–48 hours. Such snails may be kept in small containers for many days and provide an abundance of mature cercariae for live study. Later, the snail can be killed to secure the immature cercariae and earlier asexual stages of the parasite.

Much attention has recently been given to differences in number and character of the various glands which occur in cercariae. Cort (1919) studying sections of the cercaria of *Schistosoma japonicum* stained with Delafield's haematoxylin and erythrosin observed that: "The contrast between the head-gland and the cephalic glands is shown very clearly by reactions of their glandular substances to stains. The head-gland in the sections studied took the red stain (erythrosin), while the cephalic glands were stained a light blue with haematoxylin." Faust (1919) studied the cercariae of *S. haematobium* and *S. mansoni* and concluded that: "The cercariae of *Schistosoma haematobium*, *S. mansoni* and *S. japonicum* are easily distinguished on the basis of number and type of mucin glands and ducts, and their outlets." The following year (1920), he confirmed the earlier observations on the specific reaction of these glands in the human blood flukes, using Best's calcium-ammonium-carmine stain to supplement the haematoxylin-erythrosin technique. Realising that the term mucin gland formerly employed by him was not tenable, Faust (1921) suggested for these cells the use of Looss' term "cephalic gland" which had been adopted by Cort. He suggested further that, "basophilic and acidophilic glands are complementary in function and that a larva possessing the two differentiated types can penetrate the host much more effectively than one possessing only one type." Faust (1922) stated that, although present knowledge is too fragmentary to formulate any broad statement regarding the specificity of the cephalic glands in various groups of trematodes: "No organs of the cercaria save the cephalic glands and the flame cells have thus far proved satisfactory for differential diagnosis of the larva."

In the cercaria of *S. japonicum*, Faust and Meleney (1924) report that the contents of the head-gland in the mature cercaria are basophilic and that the cephalic glands, which are all of one type, are basophilic in early stages and that later they pass through a neutrophilic to an acidophilic phase. Citing differences in the observations of Cort (1919) and Faust and Meleney (1924) regarding the staining reactions of the head and cephalic glands in the cercaria of *S. japonicum*, Blacklock and Thompson (1924) point out that the head-gland of that species may be either acidophilic or basophilic, and that the contents

of the cephalic glands change from basophilic to acidophilic. They found, furthermore, that in *S. haematobium* the staining reactions are not constant even in fully developed cercariae, but differ with the state of secretory activity. These authors contend that: "The acidophilic or basophilic character of these cells is not a sufficiently definite nor stable one to justify its use as a distinguishing specific feature." Faust (1926) maintained that if sections stained with haematoxylin and countersigned with eosin are compared with others in which Best's alum carmine is used as a counter stain, the staining reactions of the cephalic glands are definite and characteristic. He stated that: "The essential differentiating features of the apharyngeal fork-tailed cercariae consist in the number and microchemical reactions of the secretory cells. These structures can be studied and differentiated from well preserved material as well as from fresh material."

Miller (1926) summarised the data pertaining to the large unicellular glands present in various cercariae and described by different authors as salivary, poison, cephalic, digestive, mucous, mucin, salivary-mucin, periacetabular, and secretory glands. He submitted evidence to show that they function as penetration glands and accordingly discarded other designations. In the brevifurcate distome larvae he found that they were differentiated "into two sorts: anterior coarsely-granular glands which are oxyphilic in sections and posterior finely-granular glands which are basophilic."

It appears that much of the difference of opinion concerning the contents of the penetration glands may be explained by the partial failure of the technique employed. Bayliss (1928) has pointed out that: "Although the terms 'acidic' and 'basic' serve to indicate an important difference between dye-salts, their careless use may lead to unwarranted conclusions. Thus, if a cell constituent takes up a basic dye, it does not follow that this body has the chemical nature of an acid....At one time it was thought to be possible to distinguish between 'basophilous' and 'acidophilous' tissue elements, according to their affinity for basic or acidic dyes...but the same substance may take up either kind of dye, according to the conditions present." This exposition may explain the reversible staining reactions observed in the penetration glands of the human blood flukes. Gatenby (1928), discussing the use of Best's alum carmine, reports that although the stain is usually successful with vertebrate tissues, "with invertebrate materials, especially from paraffin sections, even though soaked in 1 per cent. celloidin, the results are often disappointing." Actually the stain was developed as a specific technique for the identification of glycogen and its colour reaction to acids and bases is too indefinite and uncertain to regard it as a satisfactory indicator. If two test tubes, containing solutions of the stain as prepared for use, are placed in a test block and one is rendered acid while the other is rendered alkaline, the colour differences are very slight.

In the study of the penetration glands of many different types of cercariae I have employed the method recommended by Faust, sometimes with very

indifferent results. The sectioning of parasitised snails and staining of the cercariae are unsatisfactory since in immature specimens the gland cells are not functional and in older ones the staining reaction of the cells may be temporary and later reversed. Furthermore, on fixation the discrete secretory granules, so conspicuous in living specimens, are frequently decomposed so that the contents of the gland cells appear as a homogeneous mass or as a precipitate which takes the stain in a general and diffuse manner. As noted above, it is sometimes possible to recognise slight colour differences between basophilic and acidophilic cells, but the distinction is frequently not unmistakable and seldom obvious.

Cort (1919) reported that Narabayashi and Miyigawa advocated intra-vitam staining of cercariae, but that he "found no advantage in intra-vitam staining in the study of a cercaria such as that of *S. japonicum* in which the structures are clearly differentiated." Miller (1926) reported that: "Intra-vitam staining with neutral red proved very useful in the definition of certain glands and organs, and in inhibiting the activity of the larvae."

Where haematoxylin counterstained with eosin, erythrosin, or Best's alum carmine, has proved unsatisfactory for the differentiation of the contents of glands in cercariae, I have obtained uniformly good results through the use of neutral red. In the technique employed, a small drop of a one to one thousand aqueous solution of the stain was added to a drop of water containing the larvae. Almost immediately the stain penetrates into the specimens, differentiating the glands and other structures. The contents of the intestinal caeca stain a deep red, the organs are well defined and the gland cells become particularly conspicuous. The affinity of the secretory granules for the dye serves not only to give a clear and sharp delineation of these cytoplasmic inclusions, but since the stain is an indicator of the hydrogen ion concentration, the acidophilic granules appear a brilliant red whereas the basophilic granules have a yellowish to brownish colour. Actually the degree of acidity or alkalinity is here portrayed. The nuclei of the gland cells remain unstained for a long time and appear as clear vesicles in the stained cells. It is possible to count the gland cells by noting the number of nuclei, and when there are many cells overlapping each other, counts of nuclei can be made with precision when it is otherwise difficult to determine the number of cells.

In the study of mature larvae of *Cryptocotyle lingua* the penetration glands, which are apparently all of one type, are clearly outlined with neutral red and the secretory granules are large and acidophilic. The granules present in the scattered dermal glands are smaller and basophilic in reaction. In mature specimens of *C. loossi*, a brevifurcate, apharyngeal monostome, the penetration glands are divided into two clusters; an anterior, in which the secretory granules are large and acidophilic, and a posterior group in which the granules are small and basophilic.

The significance of the presence of both acidophilic and basophilic glands in certain cercariae is as yet doubtful. The suggestion of Faust (1921) that the

exudation of the two types of secretion facilitates penetration of the host does not appear to be satisfactorily supported.

Aqueous mounts of intra-vitally stained cercariae can be preserved for a considerable time if the coverglass is surrounded by a film of melted vaseline or oil to prevent the evaporation of the water. As a cercaria becomes moribund, the neutral red colour fades from the cytoplasmic structures and appears in the nuclei, reversing the earlier appearance. One may thus recognise the condition of a specimen and determine when death changes take place. In dead larvae the nuclei are deeply stained and the genital anlagen and suckers become prominent.

The use of neutral red preserves the advantages of study upon living specimens and at the same time provides excellent differential staining of the several structures. It is particularly favourable for the examination of gland cells and gives a precise and obvious measure of the acidity or alkalinity of the cytoplasmic granules in these cells. In those cercariae in which the gland cells change their reaction as development proceeds (see accounts of Faust and Meleney, and Blacklock and Thompson), the method may indicate the stage of development or of functional activity of the glands and permits a more detailed, more specific, and more complete description. For the study of fixed and preserved specimens neutral red is not a satisfactory stain. With such material much better results are obtained by the use of Delafield's or Ehrlich's haematoxylin followed by a suitable counter stain. Very good histological differentiation has been obtained with Mallory's triple stain. For cytological details I have found fixation by the fluids of Bouin or Flemming and staining with iron haematoxylin to give uniformly good results.

Since the classical researches of Looss, the excretory system of trematodes has been regarded as of major importance in any consideration of the embryology, phylogeny, or taxonomy of the group. Deep seated, fundamental, conservative, constant in form in all known genera and in many families, the system constitutes a valuable basis for comparative study. The manner of its formation, by repeated division of flame cells and ducts, tends to establish definite structural patterns. It is one of the three organ systems (excretory, digestive, and reproductive) which appear first in the cercaria and complete their development as the worm matures in the final host. The systems usually arrive at their final form in the following order: excretory, digestive, and reproductive. Frequently the excretory system is sufficiently developed in the cercarial stage that the pattern, characteristic of the group to which the adult belongs, can be recognised and the larva may accordingly be referred to its appropriate category. It has been assumed that cercariae with a common excretory pattern are closely related and that those with unlike patterns are more distantly related. Such an assumption underlies Faust's (1924) classification of the larval trematodes. While in general the idea is undoubtedly correct, too literal application may result in error. Indeed, Miller (1926) has shown that certain of Faust's groups are not homogeneous, and more recently

I have shown, Stunkard (1929), that in the family Heterophyidae the excretory system does not have a constant, uniform pattern and cannot be expressed by a simple formula as postulated by Faust.

The excretory system can best be observed in flattened, living specimens, and although it requires care, patience and frequently an abundance of living material, the flame cells and capillaries as well as the larger components of the system can usually be worked out. Although the tracing of the system is tedious and difficult, knowledge concerning it is of great value in identifying cercariae, in determining relationships, and in experimental demonstrations of life histories.

SUMMARY.

The advantages and difficulties of various methods employed in the study of larval trematodes are discussed. The desirability of using living specimens rather than fixed material and of studying mature, normally emerged cercariae rather than those obtained by crushing the host is noted. Intra-vitam staining with neutral red is recommended to demonstrate the form and reaction of the secretory granules in gland cells. Knowledge of the details of the excretory system is of major importance in both theoretical and experimental work.

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(*MS. received for publication* 17. XII. 1929.—Ed.)